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MINERAL INTERRELATIONSHIPS IN PARAKERATOSIS OF SWINE[1]

R. W. Luecke, J. A. Hoefer, W. S. Brammell and F. Thorp, Jr.[2]

Michigan State University

SINCE 1949 the authors have been confronted with periodic outbreaks of a dermatitic condition in the experimental swine herd. The same disease has also been observed under field conditions. The dermatosis has been found most frequently in pigs of from 8 to 12 weeks of age and it appeared to be similar to that described by Hogan and Johnson (1941). The gross symptoms and histopathology of this disease have been described by Kernkamp and Ferrin (1953), who termed the syndrome parakeratosis. Apparently this disease has become rather widespread since it has been noted by Brinegar (1952), Ellis (1953), Raper and Curtin (1953) and Wallace (1953). Among these investigators Raper and Curtin (1953) observed that when supplemental cobalt and zinc were added to a corn-cottonseed meal ration no symptom of the disease was observed.

In the authors own experience, repeated attempts to ascertain the etiology of the disease met with complete failure. However, since all attempts to transmit the disease to healthy pigs proved negative it was felt to be of nutritional origin.

Recently, Tucker and Salmon (1955) reported that symptoms of parakeratosis could be prevented or cured by zinc supplementation. In addition, these investigators showed that high levels of calcium and/or phosphorus markedly increased the incidence of the disease.

The objective of the experiment reported in this paper was to determine whether parakeratosis could be produced experimentally by feeding a ration high in calcium and phosphorus, and, in addition, to study the role of zinc in preventing the occurrence of the disease.

Experimental Procedure

Forty healthy weanling pigs approximately 6 weeks of age were divided into four comparable lots. All animals were sprayed for mange, wormed, and vaccinated for hog cholera; and were self-fed the various

[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 1831.

[2] Departments of Agricultural Chemistry, Animal Husbandry and Animal Pathology, East Lansing.

rations in pens with concrete floors. The duration of the experiment was 8 weeks.

The two basal rations used are shown in table 1. The crude protein, calcium, and phosphorus determinations were carried out by the usual A.O.A.C. methods (1950), and the zinc determinations were made using the one-color dithizone procedure as described by Benne (1955). Table 1 shows that ration A contained considerably more calcium and phos-

TABLE 1. COMPOSITION OF BASAL RATIONS TOGETHER WITH RELEVANT CHEMICAL ANALYSES [a]

	Ration	
	A	B
	%	%
Corn	78.5	80.0
Soybean oil meal	10.0	10.0
Meat and bone scrap	4.0	4.0
Fish meal	2.0	2.0
Dehydrated alfalfa	2.5	2.5
Limestone	1.5	0.5
Dicalcium phosphate	0.9	0.4
Iodized salt	0.5	0.5
Trace mineral premix [b]	0.1	0.1
Crude protein, %	15.1	15.2
Calcium, %	1.50	0.98
Phosphorus, %	0.80	0.70
Zinc, p.p.m.	31	29

[a] All rations were supplemented with ample amounts of vitamin A and D. In addition, all rations were supplemented with chlortetracycline at the rate of 20 gm. per ton, and a B vitamin supplement (Merck's No. 58C) at the rate of 2 lb. per ton.

[b] Calcium Carbonate Corporation.

phorus than is commonly recommended for growing swine. The National Research Council (1953) recommendations are 0.65% calcium and 0.45% phosphorus for 50 lb. pigs.

Results and Discussion

The more important results of the experiment are presented in table 2. It can be seen that the average daily gain and feed conversion of the pigs in lot 1 were extremely poor. By the end of the fourth week of the trial all 10 animals showed the typical lesions of parakeratosis with seven of the ten pigs showing very severe symptoms. The skin became thickened and heavily incrusted, with deep fissures breaking the incrusted

areas. These incrustations were most severe in the regions of the thigh, hock, ears and snout. Diarrhea was also noted in the animals most severely affected with the disease. In the remaining three pigs of lot 1, only a mild dermatosis developed, and by the end of the eighth week a very definite improvement in the condition of these three pigs was noted.

The pigs in lot 2 were fed ration A supplemented with 20 p.p.m. of zinc fed as zinc carbonate (table 2). The results of zinc supplementation were spectacular; the average daily gain of these pigs was virtually double that of the unsupplemented animals in lot 1, and by the end of the fourth week only one animal in this group developed mild symp-

TABLE 2. RESULTS OF HIGH MINERAL LEVEL AND ZINC SUPPLEMENTATION ON GROWTH RESPONSE AND INCIDENCE OF PARAKERATOSIS IN GROWING PIGS

(Ten pigs per lot on trial for eight weeks)

Lot No.	Ration and supplement	Av. initial wt.	Av. daily gain	Feed per lb. gain	No. with dermatosis
		lb.	lb.	lb.	
1.	A	27.9	0.54	3.95	10
2.	A+20 p.p.m. Zn	27.9	1.06	3.02	1
3.	B	27.9	0.91	3.23	3
4.	B+20 p.p.m. Zn	28.0	1.25	2.86	0

toms of parakeratosis. The lesions on this animal were confined to small, reddened, edematous areas on the abdomen. By the end of the eighth experimental week these lesions had healed and the animal was gaining normally. The manifestation of the disease in a mild form in this one animal suggests that the zinc level used with ration A was not completely protective against the development of parakeratosis.

The pigs in lot 3 were fed ration B, which was lower in calcium and phosphorus than ration A (table 1). The results shown in table 2 indicate that the reduction in the limestone and dicalcium phosphate level in the feed had a very favorable influence on body weight gain and feed conversion. However, although average feed-lot performance was improved very significantly over lot 1, three of the ten pigs in the group developed symptoms of parakeratosis. Of these three pigs, two developed severe symptoms of the disease.

The pigs in lot 4 received ration B supplemented with 20 p.p.m. of zinc, as zinc carbonate. No symptom of parakeratosis was noted in any animal in this group, and the average daily gain and feed conversion

were excellent. These results indicate that for ration B the supplemental zinc level used was sufficient to protect against symptoms of parakeratosis.

The effect of zinc therapy on parakeratosis was studied by placing all of the pigs from lot 1 on ration A supplemented with 20 p.p.m. of zinc at the conclusion of the eighth week. Improvement in appetite was noted within a few days. During the first eight weeks of the experiment (table 2) the pigs in lot 1 averaged only 0.54 lb. per day; however, during the subsequent six weeks of zinc therapy the average daily gain for the same group was 1.54 lb. Nevertheless, it required from 3 to 6 weeks for the skin lesions to regress and return to normal.

TABLE 4. ZINC CONTENT OF SOME COMMONLY USED FEED INGREDIENTS [a]

Ingredient	Zn
	p.p.m.
Soybean oil meal (solvent)	77
Cottonseed oil meal (expeller)	66
Cottonseed oil meal (solvent)	63
Meat and bone scrap	131
Fish meal (Menhaden)	64
Alfalfa meal (dehydrated)	32
Corn	31
Oats	34
Bone meal	146
Limestone (38% Ca)	4
Dicalcium phosphate (26% Ca, 21% P)	0

[a] Average of results from duplicate determinations by the one-color dithizone procedure of Benne (1955).

The results indicate that an elevated mineral level in the feed is a predisposing factor in swine parakeratosis. Whether a high calcium level is the only factor is not known. Tucker and Salmon (1955) suggested that the level of calcium or phosphorus or both are contributing factors in the incidence of the disease. However, it may well be that an increased amount of calcium in the ration may intensify the need for dietary zinc. In this connection, it is interesting that in the calf an elevated calcium level in the feed causes an increase in the animal's requirement for dietary manganese (Hawkins *et al.*, 1955).

Because there is very little information available on the zinc content of commonly used swine feed ingredients, the results of zinc analyses of some of these ingredients are given in table 3. These data were obtained on single samples of each ingredient, and thus do not indicate the variation which might be present in different batches of the same feedstuff.

It is interesting that meat and bone scrap and bone meal contain considerably more zinc than do the other feedstuffs analyzed. It may be that since plant protein concentrates are being used in increasing amounts to replace animal proteins in practical swine rations, the zinc content of these rations has been decreasing. This would also result from the increasing use of limestone, dicalcium phosphate and other mineral ingredients in place of bone meal.

From a practical standpoint, the results of this experiment point to the need for a careful consideration of calcium and phosphorus levels in the formulation of swine rations. Also the need to study the role of zinc in its relationship to calcium and parakeratosis is readily apparent.

Summary

In an experiment involving 4 lots of 10 pigs each, 100% incidence of parakeratosis was produced by feeding a ration containing 1.50% calcium, 0.80% phosphorus and 31 p.p.m. zinc. When the same ration was supplemented with 20 p.p.m. of zinc (as zinc carbonate) only one animal of the group developed mild symptoms of the disease.

Parakeratosis was produced in three of ten pigs by feeding a ration containing 0.98% calcium, 0.70% phosphorus and 29 p.p.m. zinc. Parakeratosis was completely prevented in 10 pigs by the supplementation of this latter ration with 20 p.p.m. of zinc.

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THE EFFECTS OF A MANGANESE DEFICIENCY UPON THE GROWTH, DEVELOPMENT, AND REPRODUCTION OF SWINE [1]

M. P. PLUMLEE, D. M. THRASHER, W. M. BEESON, F. N. ANDREWS AND H. E. PARKER

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THE effects of a dietary deficiency of manganese upon poultry and laboratory animals, such as rats, mice, and rabbits, have been known for several years. Wilgus, *et al.* (1936) demonstrated that perosis could be prevented in chicks by adding manganese to a perosis-producing diet. Among others, manganese deficiency symptoms such as congenital debility, reproductive failures, ataxia, shortened and crooked bones, and reduced bone-breaking strength have been observed by several workers in the laboratory animals.

Miller *et al.* (1940) reported that natural rations may be deficient in manganese for swine. Thirty of 60 pigs developed lameness at 150 lb. liveweight when fed a natural ration containing 11 to 14 p.p.m. of manganese. The addition of manganese to supply 50 to 60 p.p.m. in the ration prevented but did not cure the condition. Johnson (1943, 1944) reported satisfactory growth in pigs fed both natural and semi-purified rations containing low levels of manganese, but no lameness was observed. However, Grummer *et al.* (1950) noted a significantly improved growth rate in pigs fed a ration composed of natural feedstuffs containing 52 p.p.m. manganese compared to pigs on the basal ration alone (12 p.p.m. manganese). The gilts from the basal group required a higher number of services per conception and farrowed fewer pigs per litter.

The series of four experiments herein reported were undertaken to determine the quantitative requirement of swine for manganese, to characterize the symptoms of a manganese deficiency in growing and reproducing boars and gilts, and possibly to establish a function of manganese in this species.

[1] This research was supported in part by an endowment from the Lilly Research Foundation, Eli Lilly Company, Indianapolis, Indiana.

[2] Contribution from the Departments of Animal Husbandry and Biochemistry, Journal Paper No. 926, Purdue University, Lafayette, Indiana.

Experimental

Experiment I

During the summer of 1950, nine weanling Duroc boar pigs (35 lb. average initial weight) were hand fed three levels of manganese (1.5, 20.5, and 34.0 p.p.m.) in a purified ration composed of commercial casein, 22.4; cerelose, 65; corn oil, 5; celluflour, 2; mineral mixture, 5.2; vitamin mixture, 0.26; and whole liver powder 0.50. The mineral and vitamin fortifications were essentially the same as listed in table 1

TABLE 1. THE COMPOSITION OF THE BASAL RATIONS FOR EXPERIMENT III

Ingredient	Basal "A"	Basal "B"
Yellow corn	53.53	
Cerelose		38.58
Dried skimmilk	40.60	54.55
Celluflour	1.00	3.00
Corn oil	2.00	1.00
Whole liver powder	0.50	0.50
Profactor B, 10 mg. vit. B_{12} per lb.	0.10	0.10
Mineral mixture [a]	2.00	2.00
Vitamin carrier mixture [b, c]	0.27	0.27
	100.00	100.00

[a] Mineral mixture (Mn-free)

Ingredient	%
Dicalcium phosphate	46.320
Calcium carbonate	16.900
Sodium chloride	13.500
Magnesium carbonate	8.500
Potassium phosphate	5.700
Potassium chloride	6.700
Iron pyrophosphate	2.100
Copper sulfate	0.020
Zinc oxide	0.016
Cobalt carbonate	0.016
Potassium iodide	0.228
	100.000

[b] Vitamin carrier mixture

	gm.	mg. per lb. feed
Thiamine	2.50	1.0
Riboflavin	4.00	1.6
Niacin	25.00	10.0
Calcium pantothenate	22.50	9.0
Pyridoxine	3.00	1.2
Choline chloride	1125.00	450.0
Vitamin K	5.00	2.0
Folic acid	0.75	0.3
Total	1187.75	
Casein	1812.25	
Total mix	3000.00	

[c] Thirty-five grams of a Vitamin A and D oil concentrate containing 3000 I.U. of A and 400 I.U. of D per gram, and 10 gm. of alpha-tocopherol were added per 100 lb. of total ration at the time of complete mixing.

for Experiment III. The animals were maintained on raised wire screen platforms in individual pens and fed in metal feeders for the entire 14 weeks of the trial. All drinking and wash water was treated with zeolite softener which removed an average of 87% of the manganese present in the water. The "soft" water contained 0.014 p.p.m. manganese by analyses. Average daily gains for the 1.5, 20.5 and 34.0 p.p.m. manganese treatments were 1.58, 1.62 and 1.71 lb. per day, respectively. These differences were not significant. No gross manifestation of bone deformities or abnormal gaits was noted in any of the pigs. Gross and microscopic examination of several glands and organs revealed no marked differences in physiological structure of tissues from pigs on the three manganese levels. The manganese content of the livers varied directly with the dietary manganese levels, being an average of 2.2, 10.4, and 12.3 p.p.m. on a dry-weight basis for the low, medium and high treatments, respectively. Scrapings from the metal troughs were analyzed and found to contain approximately 900 p.p.m. manganese. No doubt there was some contamination of the rations from these troughs.

Experiment II

In this trial two lots of 6 weanling Duroc boar pigs each were individually fed from an average initial weight of 43 lb. to an average final weight of 246 lb. on diets containing either 1.4 or 40 p.p.m. manganese. The basal ration was similar to the one used in Experiment I except that the liver powder was omitted. Otherwise the same feeding and management practices were followed as before. Growth rate and feed efficiency were excellent with no significant difference between the two groups. Each lot gained an average of 1.7 lb. per pig daily on an average of 3.1 lb. of feed per pound of gain. The manganese contents of the liver, heart, thyroid and testes averaged 4.6, 0.65, 0.37 and 0.99 p.p.m., respectively, on a dry-weight basis for the animals on the low manganese (1.4 p.p.m.) treatment, and 12.1, 1.53, 0.91 and 2.69 p.p.m., respectively, for those on the 40 p.p.m. manganese level. This is a much smaller difference than occurred in the first experiment and may have been due to the greater initial manganese stores in the larger pigs which were used. The manganese content of the blood varied little between animals or treatments, averaging 5.95 and 6.96 mcg. per 100 ml. of blood for the 1.4 and 40. p.p.m. manganese groups, respectively. Gross examination of the animals indicated a tendency toward a "sickle-hocked" condition in the pigs fed the 1.4 p.p.m. level of manganese.

Experiment III

Procedure. Four groups of 4 Duroc gilts each were individually hand fed rations containing 1.0, 3.4, 38, and 40 p.p.m. of manganese from weaning throughout the gestation and lactation periods. The animals were maintained in concrete-floored pens with wood panels and were fed and watered twice daily in glass-lined, metal troughs to minimize contamination of the ration with manganese. Again, "softened water" was provided that contained only traces of manganese. The two basal rations, "A" and "B," containing 3.4 and 1.0 p.p.m. manganese, respectively, are given in table 1. Basal "A" plus manganese contained 40 p.p.m. total manganese, and basal "B" plus manganese contained 38 p.p.m. Manganese chloride was used to supply the additional manganese to each basal.

In addition, the reproductive performance of the second-generation boars from two of the gilts fed the low-manganese rations was studied.

Determinations of tissue manganese, blood plasma alkaline phosphatase (method of King and Armstrong, 1934; modified by Wiese *et al.*, 1939), and calcium and phosphorus content of hock bones were made on the gilts. The liver and heart from several of the pigs were analyzed for total manganese. Histological examinations of the testes from the second-generation boars were made.

The absorption, excretion and tissue distribution of orally administered Mn^{54} were studied in two second-generation low-manganese boars and two control barrows. The boars were farrowed by a gilt that received the basal "B" ration (1.0 p.p.m. Mn) and were raised on their dams' milk plus a similar ration that contained 3.5 p.p.m. manganese. Each pig weighed approximately 140 lb. and was dosed by a stomach tube with 0.5 millicurie of Mn^{54} in the form of manganese chloride. Twice daily separate urine and feces collections were made until all appreciable radioactivity had disappeared from the urine and feces. This period was 96 hours for one pig on each treatment and 120 hours for the other two.

Results. Growth rate and feed efficiency of the gilts were very good on all treatments, and there was no observed difference attributable to the level of manganese in the diet. The average daily gain for all 4 lots to 250 lb. was 1.7 ± 0.07 lb.; feed per 100 lb. gain varied from 350 to 380 lb. No difference in number of pigs farrowed, birth weight, or livability could be correlated with the levels of manganese in the diets. Total manganese in the soft tissues of both gilts and their pigs was reduced about 50% by the prolonged feeding of low-manganese rations

as shown in table 2. No difference in organ weights, blood phosphatase or phophorus and manganese content of the hock bones was observed. The plasma alkaline phosphatase values taken two months after the start of the experiment averaged 10.5, 10.7, 11.2 and 14.5 units for the 1.0, 3.4, 38 and 40 p.p.m. manganese treatments, respectively. A unit is defined as the number of milligrams of phenol liberated by 100 ml. of plasma under the conditions described by King and Armstrong (1934). No other analysis for phosphatase was made. Second-generation boar pigs grew well on basal "A" and showed normal spermatogenesis.

TABLE 2. THE MANGANESE CONTENT OF SEVERAL TISSUES FROM THE GILTS AND THIRTY-ONE OF THEIR PIGS, (p.p.m.)

	Gilts					Pigs[a]		
Treatment	Liver	Heart	Spleen	Kidney	Hock bone	No. analyzed	Liver	Heart
Basal "A" (3.4 p.p.m. Mn)	4.17	0.64	1.11	3.00	1.42	10	5.45	0.95
Basal "A" + 36.6 p.p.m. Mn	9.07	1.25	1.30	4.29	1.46	16	9.57	1.91
Basal "B" (1.0 p.p.m. Mn)	2.81	0.60	0.88	3.52	1.31	3	6.74	.90
Basal "B" + 39 p.p.m. Mn	11.33	1.53	1.45	6.1	1.64	2	8.80	2.46

[a] All of the pigs analyzed were either stillborn or died within 14 days after birth.

For the control animals the average percentage of the dose of Mn^{54} excreted in the feces and urine was 83 and 0.35, respectively. The corresponding values for the low-manganese boars were 78 and 0.28. The difference was reflected by increased concentrations of Mn^{54} in the liver, kidney, and pancreas of the animals on the low-manganese diet.

Experiment IV

Procedure. Twenty crossbred suckling female pigs, about three weeks of age and averaging 9.4 lb., were equally divided into two groups on the basis of weight, littermates and general appearance and were fed a semi-purified ration containing either 0.5 or 40 p.p.m. manganese throughout the growth, gestation, and lactation periods. Basal "B," shown in table 1, was used with the following modifications: 36.58% cerelose, 3.00% corn oil, 13.5% iodized salt replacing salt and potassium iodide in the mineral mixture, 2.328% $FeSO_4 \cdot 7H_2O$ replacing iron pyrophosphate in the mineral mixture, and manganese sulfate to supply additional manganese in place of manganese chloride. In addition, oxytetracycline was incorporated into the diet at the rate of 10 mg. per

pound during the second week of the experiment to control scouring and was continued in the ration until the end of the growth period. The gilts on each treatment were fed in groups of five until a few days before farrowing. Lots 1 and 2 received the basal ration containing 0.5 p.p.m. manganese, while lots 3 and 4 were fed the basal diet plus enough additional manganese to bring the level to 40 p.p.m. The same types of pens, feeders, waterers, and water used in Experiment III were employed in this trial.

The level of celluflour in the diet was increased to 5% when the pigs averaged about 50 lb., and the level of protein was decreased periodically as the pigs gained in weight.

Feed consumption and growth data were calculated on a weekly basis throughout the growth period, which was terminated when the gilts averaged approximately 194 lb. The ration shown in table 3, which contained about 24.5% protein, was fed until the pigs reached an average weight of 30 lb., at which time they were changed to the ration previously described.

During the last 9 weeks of the growth period the supplemental choline chloride was omitted from the basal ration of one lot of 5 gilts on each manganese treatment. Near the end of the growth period, 117th day of experiment, direct backfat measurements were taken on all gilts, and radiograms of the legs of four gilts were made. On the 122nd day of experiment, one gilt from each treatment was sacrificed to obtain bone and tissue samples for chemical and histological studies.

At the end of the growth period the gilts were placed on a restricted feeding regime to prevent them from becoming overfat. The vitamin and mineral concentration was increased 50% and the daily intake was held to 5 lb. per gilt. At 8 months of age they were hand-mated to two fertile boars, and those that did not settle or were not bred were slaughtered in an attempt to determine the cause of reproduction failure. Following farrowing, samples of colostrum and milk were obtained from one high-manganese and two low-manganese sows, and several pigs were sacrificed to obtain tissues for total manganese analyses. The other pigs were raised on the sow, having free access to her ration, and were weaned at 6 weeks of age. All sows were slaughtered to obtain samples of the liver, heart, kidney, and spleen. These and all other samples were analyzed for total manganese by the microcolorimetric method of Gates and Ellis (1947).

Composite feed and water samples were taken throughout the experi-

ment and were analyzed each four to six weeks for total manganese. The average manganese contents of the basal ration, basal plus manganese, and the "soft" water were <1.0, 40.8, and 0.06 p.p.m. respectively.

TABLE 3. COMPOSITION OF THE BASAL DIET USED UNTIL THE PIGS REACHED 30 LB. LIVEWEIGHT [a]

Ingredient	%
Dried skimmilk	72.17
Cerelose	11.64
Celluflour	2.00
Corn oil	6.00
Whole liver powder	0.50
Potassium phosphate	1.56
Calcium phospate	2.63
Iodized salt	0.72
Calcium carbonate	1.39
Profactor B, 10 mg. vit. B_{12} per lb.	0.15
Trace mineral mix [b]	0.35
Vitamin A and D oil [c]	0.62
Vitamin carrier mix [d]	0.27
	100.00

[a] Terramycin HCl added at the rate of 10 mg. per lb. during the second week of the experiment and was continued until the end of the growth period.

[b] Trace mineral mix

	gm.
$MgSO_4$	97.68
$FeSO_4 \cdot 7H_2O$	53.33
$CuSO_4$	0.59
Zn 0	0.23
CaF_2	0.98
$CoCO_3$	0.20
	153.01

[c] Contains 3000 IU of Vit. A and 400 IU of Vit. D per gram.

[d] Vitamin carrier mix

	gm.	mg. per lb. feed
Mixed tocopherol	7.95	3.18
2-methyl naphthoquinone	2.06	0.83
Thiamine	55.50	22.20
Riboflavin	10.00	4.00
Niacin	75.00	30.00
Calcium pantothenate	60.00	24.00
Pyridoxine	10.00	4.00
Choline chloride	1000.00	400.00
Para-aminobenzoic acid	20.00	8.00
Biotin	0.08	0.03
Inositol	100.00	40.00
Folic acid	10.00	4.00
Ascorbic acid	125.00	50.00
Total vitamins	1475.59	
Total	3000.00	
Casein	1524.41	

Results

The Growth Period. The growth rate and feed-consumption data are summarized in table 4. The removal of the supplemental choline chloride from the ration of half the gilts on each manganese treatment did not hasten the development of manganese deficiency symptoms, but the gilts receiving no supplemental choline had a non-significantly faster rate of gain. The supplemental choline was removed from the basal ration at the end of the growth period. The small differences in growth rate and feed efficiency between the two manganese treatments were not significant. Considering the age of the pigs at the start of the experiment, the growth rate and feed efficiency were very satisfactory.

TABLE 4. GROWTH RATE AND FEED CONSUMPTION OF GILTS FED RATIONS CONTAINING 0.5 AND 40.0 p.p.m. MANGANESE

Item	Lot number					
	1	2	3	4	1 and 2	3 and 4
Mn in ration, p.p.m.	0.5	0.5	40.0	40.0	0.5	40.0
No. animals						
Initial	4[a]	5	5	5	9	10
Final	3[b]	5	4[b]	5	8	9
Av. initial wt., lb.	9.2	9.7	9.8	8.9	9.5	9.4
Av. final wt., lb.	184.8	198.1	188.5	199.5	193.1	194.6
Av. daily gain, lb [c]	1.36	1.39	1.35	1.43	1.38	1.39
Av. daily feed, lb.	4.35	4.28	4.26	4.27	4.31	4.26
Feed per lb. gain, lb.	3.20	3.08	3.14	2.98	3.13	3.06

[a] One small pig from lot 1 died the second day of the experiment.
[b] A gilt from each of these lots was sacrificed on the 122nd day for bone and tissue analyses.
[c] Calculated by dividing gain by pig days.

The development of manganese deficiency symptoms began to appear in the low-manganese gilts at an average weight of approximately 55 to 60 lb. These gilts would occasionally sit on their buttocks while eating. Gradually, the condition grew worse, and shifting of the hind feet while standing was noted. In addition, several other changes slowly developed in the low-manganese animals that were not present in the high-manganese gilts. Shorter and thicker front legs were noted at about 90 lb., and two or three gilts were beginning to exhibit bowed front legs. From 135 lb. up, several low-manganese gilts appeared to have enlarged hocks, reduced body length and height, weaker pasterns, appeared to be fatter, and were less active than the high-manganese gilts. All the gilts on the low-manganese ration showed excess fatness, reduced body length and height, and shorter legs, but only about one-

half exhibited bowed legs or enlarged joints. These symptoms suggest that general skeletal development was retarded and that fat deposition was increased. Figure 1 shows some of the symptoms previously noted and compares a typical gilt from the 40 p.p.m. manganese lot to a littermate from the 0.5 p.p.m. manganese lot.

The direct probe method of Hazel and Kline (1952) was used to determine backfat thickness. The average of three positions along the back was taken as the backfat thickness for each gilt. The average backfat thicknesses for the low- and high-manganese treatments were 1.62

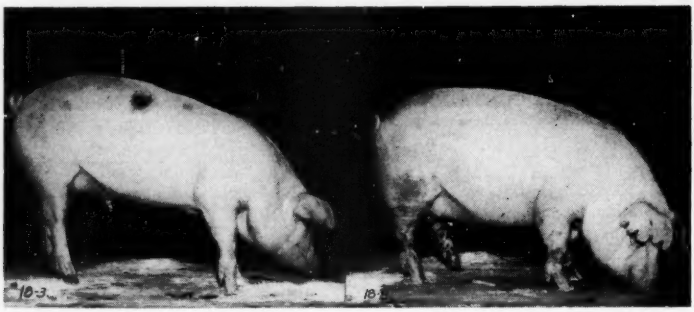

Figure 1. Gilt 18–3, left (40.0 p.p.m. Mn), weight 164 lb., age 132 days. Gilt 18–8, right (0.5 p.p.m. Mn), weight 174 lb., age 132 days a littermate to gilt 18–3. Note excessive fatness.

and 1.42 in., respectively, the difference being statistically significant ($P<0.05$). The gilts on each treatment averaged approximately 164 lb. at this time.

Radiograms of the legs of three low-manganese gilts revealed several bone changes not present in radiograms of the legs of a high-manganese animal. A definite shortening of the radius, ulna, metacarpals, tibia, fibula, and metatarsals could be seen. The distance between the distal epiphyseal plates of the radius and ulna was considerably shortened, and widening of the diaphysis had occurred adjacent to the distal epiphyseal plate of the ulna. Also, an extensive rarefied area 1 to 2 centimeters proximal to the distal epiphyseal plate of the ulna could be seen. In addition to these findings closure of the distal epiphyseal plate of the radius was taking place, while the adjacent ulnar epiphyseal plate remained open. Since the ulna was capable of continued growth while the radius was not, the disproportionate growth of these bones could account in part for the bowed front legs in some gilts. The bones from

the two gilts that were sacrificed also showed the above differences. The femur from the low-manganese gilt was shorter by 1.5 in. The details of this phase of the study are presented in another paper.

The histological study of the liver, spleen, pituitary, thyroid, adrenals, and reproductive organs from the two sacrificed animals revealed that these tissues from both gilts were completely normal. A longitudinal section of a radius and an ulna from each gilt showed the same differences as seen in the radiograms.

Breeding and Gestation. During the breeding period the high-manganese gilts exhibited more regular estrual cycles than the low-manganese gilts. Two gilts in the latter group were never observed in estrus, while a third did not come into estrus until 2 days before the breeding period ended. A fourth gilt appeared to be in estrus regularly but would not accept the boar. Two low-manganese gilts had some difficulty in supporting the boar, and three gilts that were bred did not come into estrus again and did not farrow. One low-manganese gilt that did not farrow showed definite evidence of fetal death and resorption upon examination of her reproductive tract. Examination of the ovaries of the gilts that did not come into estrus revealed that ovulation was occurring. Of the 8 low-manganese gilts, 5 were bred and only 2 farrowed; the group required 2 services per conception. Of the 9 high-manganese animals, 9 were bred and 8 farrowed. This group required 1.75 services per conception.

Farrowing and Lactation. Table 5 summarizes the data on the 10 gilts that farrowed. With the possible exception of gilt 20–3, all the high-manganese gilts farrowed strong litters, and even the small pigs from these litters were active and appeared normal. All these gilts exhibited good udder development prior to farrowing and came into milk normally. Sow 18–1 went off feed 4 days after farrowing due to some undetermined cause and died 18 days later. Her litter was raised on a commercial milk replacer and the ration shown in table 3. This group of sows raised their litters satisfactorily.

In contrast, the performance of the two low-manganese gilts at farrowing was entirely unsatisfactory. Prior to farrowing these gilts showed very little udder development and at parturition gave birth to very weak litters and did not come into milk normally. All of the pigs were small and weak, and showed poor balance and coordination. This was manifested by their turning in circles, falling over, bracing their feet in attempts to stand, carrying their heads to one side, and inability to nurse. Figure 2 illustrates some of these symptoms. The litter of 7 pigs

TABLE 5. A SUMMARY OF THE FARROWING AND LACTATION DATA ON ALL GILTS

Gilt No.	p.p.m. Mn	No. farrowed		Stillborn		Strong pigs		Av. birth wt. lb.	Av. weaning wt., 6 wk.	Pigs weaned	
		Total	Live	No.	%	No.	%			No.	%
								lb.	lb.		
19–1	40.0	9	8	1	11.1	7	87.5	2.6	24.1	4 [b]	50.0
17–4	40.0	8	8	0	0	8	100	2.6	25.4	8	100
19–10	40.0	12	12	0	0	11	91.7	3.2	26.2	10	83.3
17–10	40.0	11	10	1	9.1	7	70.0	2.0	17.6	8	80.0
17–3	40.0	5	4	1	20.0	4	100	2.8	24.3 [c]	4	100
20–3	40.0	10	8	2	20.0	7	87.5	1.9	14.6 [c]	8	100
18–1 [a]	40.0	11	11	0	0	11	100	2.9	19.0	9 [d]	81.8
19–7	40.0	8	8	0	0	8	100	3.5	25.1	8	100
Average		9.25	8.62	0.62	6.7	7.88	91.4	2.67	21.8	7.38	85.5
19–6	0.5	7	7	0	0	0	0	2.1	....	0	....
19–3	0.5	4	4	0	0	0	0	1.4	13.5	1 [d]	25.0
Average		5.5	5.5	0	0	0	0	1.85	13.5	0.5	9.1

[a] Died 12-15-54.
[b] Four pigs were sacrificed from this litter during the first week.
[c] Made poor gains during 1 week due to scours.
[d] Raised on a milk replacer and ration in table 3.

all died within one week due to lack of milk and their inability to nurse properly. The litter of four was fed a milk replacer containing 40 p.p.m. manganese and only 1 pig survived. The symptoms of incoordination showed by this pig gradually regressed and disappeared at approximately 8 weeks of age.

The legs of one high-manganese and three low-manganese pigs, all less than one week of age, were studied radiographically, but no difference in bone length, density, or degree of ossification was noted.

Figure 2. Litter 6 from sow 19–3 (0.5 p.p.m. Mn) showing weakness and poor sense of balance at birth.

Tissue and Milk Analyses. The total manganese content of the soft tissues of gilts was greatly reduced by feeding a ration containing 0.5 p.p.m. manganese compared to one containing 40 p.p.m. The average manganese contents of the livers, kidneys, hearts, and spleens of the 9 low-manganese animals were 1.3, 1.4, 0.5, and 0.7 p.p.m., respectively. The corresponding values for the high-manganese groups were 11.4, 9.3, 1.8, and 1.6 p.p.m. The average manganese contents of the livers, kidneys, hearts, and muscle of the 7 low-manganese pigs were 2.5, 2.1, 0.5, and 0.4 p.p.m., while 7 high-manganese pigs averaged 8.9, 5.8, 1.9, and 1.2 p.p.m. showing that the depletion in the sow was also present in her pigs at birth.

Five colostrum and milk samples taken from sow 19–1 (40 p.p.m.) averaged 0.38 p.p.m. manganese on a dry basis compared to an average of 0.27 p.p.m. for a total of three samples from the two low-manganese

sows. These two sows were milking so poorly that no more samples could be obtained.

Discussion

The growth rate and feed efficiency of swine fed from a weight of 30 to 43 lb. to varying market weights on rations containing from 1.0 to 1.5 p.p.m. manganese were very good. There was no sex difference in response noted in these three experiments. This is in agreement with the work of Johnson (1943, 1944). However, when a similar diet containing only approximately 0.5 p.p.m. manganese was fed to pigs weaned younger and smaller (age 3 weeks, wt. 9.4 lb.) normal growth did not occur. Even though the average daily gains to 195 lb. were almost identical for the 0.5 and 40 p.p.m. manganese treatments, it was obvious rather early in the experiment that the low-manganese animals exhibited reduced skeletal growth and a marked increase in fat deposition. Since all animals were similarly affected, it appears that manganese is essential for normal skeletal growth in swine. Whether the increased fattening was a primary or secondary effect of insufficient manganese is not known. The question naturally arises as to what the effect on daily gain would have been if the rations had been fed *ad libitum* to a much larger weight, e.g. 300 lb., rather than to 194 lb. Would they have overcome their deficiencies? The answer appears to be no, because the changes appeared to be permanent and were still very noticeable at the end of the experiment. Therefore, if *ad libitum* feeding had been prolonged there would probably have been a decrease in gains in the low-manganese gilts.

The effect of using young pigs with small initial weights on the occurrence of manganese deficiency symptoms cannot be over-estimated. In the first three experiments the pigs weighed from three to four and one-half times more, and assumed to have an equally greater body store of manganese at the time they were put on deficient rations, than those in the last experiment. Perhaps this additional store of manganese was sufficient to prevent any occurrence of a deficiency in the first three experiments.

The feed efficiency was very good for all treatments in all experiments and no treatment difference was noted. However, the efficiency of the eight second-generation low-managenese boars from experiment three was most marked. They required an average of 2.6 lb. of feed per pound of gain from a weaning weight of 32 lb. to a final weight of approximately 200 lb.

The leg weaknesses noted in the low-manganese gilts in experiment four did not result in lameness as was observed by Miller *et al.* (1940) and Keith *et al.* (1942), but the crooked legs and enlarged hocks noted by these workers may have been quite similar to the leg abnormalities that were observed in this study. Some lameness occurred in the first-generation gilts in experiment three of this study but it was common to all four treatments. About half of the concrete floor in each pen was covered with a wood platform in experiment four and no lameness occurred.

In contrast to this work, Johnson (1943) did not observe any abnormal pig from sows raised on a diet containing 0.5 p.p.m. manganese, but he also did not observe any evidence of a manganese deficiency in the growing gilts.

In many respects the deficiency symptoms noted throughout Experiment IV compare very closely with those that have been reported in poultry, rats, and rabbits. Shortened and crooked legs have been reported by several workers in these species, the work of Ellis *et al.* (1947) with rabbits being one example. Reports of reproduction failures in rats are also similar to the observations made in this experiment. One of the striking similarities is the report of Hill *et al.* (1950) in which ataxic rats were produced from manganese-deficient females. Their description of these rats is almost identical with the observations made on the baby pigs from the deficient gilts in this study.

Further evidence that a manganese deficiency was produced in the 0.5 p.p.m. group is revealed in the tissue analyses. Johnson (1943) reported a similar degree of depletion in pigs on a level of 0.5 compared to 100 p.p.m. manganese.

Since the average swine ration contains over 20 p.p.m. manganese it is most unlikely that such a deficiency will occur under natural conditions unless perhaps it is caused from some interfering dietary substance.

Summary

Four experiments were conducted to determine the effect of low-manganese rations on growth, fattening, and reproduction in swine.

No difference in rate of gain or feed efficiency was observed in pigs fed from a starting weight of from 9.5 to 43 lb. to a final weight of from 185 to 246 lb. on semi-purified rations containing from 0.5 to 40 p.p.m. manganese.

Second generation boars from gilts raised on rations containing either 1.0 or 3.4 p.p.m. manganese grew normally and showed normal sperma-

togenesis when raised on a ration containing 3.3 p.p.m. manganese. A study was made of the absorption, excretion, and tissue distribution of orally administered Mn^{54} by these boars.

The prolonged feeding of rations containing 0.5 to 3.5 p.p.m. manganese resulted in a tissue manganese depletion of from 2 to 10 times that of the controls receiving approximately 40 p.p.m. manganese. The greatest depletion was in the livers of the Experiment-IV gilts.

When female pigs were weaned at three weeks of age and at an average weight of 9.4 lb. and were fed throughout the growing, gestation, and lactation period on a ration containing either 0.5 p.p.m. or 40 p.p.m. manganese the following deficiency symptoms developed in the 0.5 p.p.m. manganese group: (a) reduced skeletal growth and an accompanying skeletal or muscular weakness; (b) increased fatness as shown by a statistically significant greater back fat thickness at an average weight of 164 lb.; (c) irregular estrual cycles; (d) complete absence of estrus in some animals and absence of marked signs of estrus in others; (e) resorption of fetuses or birth of small, weak pigs which could neither stand nor walk properly, with tissues containing approximately 33% as much total manganese as did those of the control pigs; (f) poor udder development and almost complete absence of milk production and secretion, the colostrum and milk containing approximately 33% less manganese than similar samples from a control gilt; and (g) a reduction in the tissue manganese in the first-generation gilts to from 10 to 20% of the controls.

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STUDIES ON TRICHLOROETHYLENE-EXTRACTED FEEDS. IX. EXPERIMENTS WITH SWINE FED TRICHLOROETHYLENE-EXTRACTED SOYBEAN OIL MEAL [1-4]

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A LARGE proportion of the total quantity of soybean oil meal produced in the United States is used in rations for swine. If this species, like the bovine, were susceptible to intoxication by trichloroethylene-extracted soybean oil meal (TCESOM) it would be expected that while this product was produced and sold commercially cases of intoxication in swine would have been noted coincident with those observed in cattle. (For review of the pertinent literature see Pritchard *et al.* 1952). Since cases of TCESOM toxicity in swine have apparently not come to the attention of veterinarians it appears that this species is much less susceptible than the bovine. This is also indicated by the few published reports of experiments in which TCESOM has been fed to swine. Stockman (1916), who made the original observations on TCESOM toxicity in cattle, fed what was presumably a toxic specimen to 2 pigs; one consumed 27 lb. and the other a total of 263 lb. but neither showed any ill effects from this treatment. Becker *et al.* (1953) fed a specimen of TCESOM of unknown degree of toxicity for the bovine (but produced in a plant which has made toxic TCESOM) at a 21.5% level to growing swine as a component of a ration containing corn, alfalfa meal and pasture. Up to 100 lb. of body weight the TCESOM supported weight gains that were about equal to those produced by hexane-extracted soybean oil meal. Between 100 and 200 lb. the weight gain of the pigs consuming TCESOM was somewhat inferior

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[3] A cooperative study between the Department of Animal Husbandry, the School of Veterinary Medicine and the Department of Agricultural Biochemistry, Institute of Agriculture, University of Minnesota.

[4] The biochemical analyses were performed by N. S. Mizuno, whose devoted assistance is gratefully acknowledged. Glen Swartz and associates fed and cared for the pigs.

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and in a protein efficiency test it was not nearly as effective as the two specimens of hexane-extracted soybean oil meal with which it was compared. The TCESOM, however, did not appear to have any evident toxic properties for swine.

Wilcke (1952) using a specimen of TCESOM which was toxic for cattle observed no harmful effects on swine during a 40-day trial.

In conjunction with other studies conducted at the Minnesota Agricultural Experiment Station it was considered desirable to test the effects on swine of a specimen of known high degree of toxicity to the bovine in experiments covering growth and reproduction of the animals as well as the growing period of the F_1 generation.

Experimental

The TCESOM available for these studies was procured from remaining stocks on a farm where cases of acute, fatal aplastic anemia in the bovine had been attributed to the consumption of this meal. It was produced in the fall of 1950 from 1950-crop soybeans and was shown in controlled experiments (Pritchard *et al.* 1956) to have a high degree of toxicity for the bovine. Two groups, each composed of six weanling purebred Chester White gilts were fed practical rations containing either hexane-extracted soybean oil meal (Lot 1) or TCESOM (Lot 2) throughout the growing period. After 5 months two pigs in each lot were slaughtered and subjected to post mortem examination. The remaining 4 pigs were retained for breeding. From the pigs of their litters six animals were selected from each lot and fed rations containing the same specimen of soybean oil meal as fed to their dams until they reached market weight of about 210 lb. This study therefore covered the growing period of a parent and of the F_1 generation and one reproductive period of the parent generation.

The rations used and the time intervals during which they were fed are shown in tables 1 and 2.

Results and Discussion

The data in tables 3 and 4 summarize the weights and weight gains of the parent generation throughout the periods of growth and reproduction. The TCESOM supported slightly slower weight gains than the hexane-extracted soybean oil meal but in both cases the rate of gain was satisfactory. The smaller weight gains of the pigs of lot 2 were associated with a smaller feed consumption so that the efficiency of feed utilization was essentially the same in both lots, as shown in table 5.

TABLE 1. COMPOSITION OF RATIONS

	Ration No. (lb.)							
	H1	T1	H2	T2	H3	T3	CH	CT
Ground yellow corn	365.0	365.0	100.0	100.0	100.0	100.0	60.0	60.0
Rolled oats	.....	.....	.....	.....	.....	.....	30.0	30.0
Ground oats	.....	.....	340.0	340.0	240.0	240.0	.....	.....
Soybean oil meal, hexane-extracted [a]	100.0	.....	50.0	.....	50.0	.....	9.5	.....
Soybean oil meal, TCESOM [b]	.....	100.0	.....	50.0	.....	50.0	.....	9.5
Alfalfa meal, dehydrated	25.0	25.0	.....	.....	.....	.....	.....	.....
Alfalfa hay, ground	.....	.....	.....	.....	100.0	100.0	.....	.....
Steamed bone meal	7.5	7.5	7.5	7.5	7.5	7.5	.....	.....
Trace mineralized salt	2.5	2.5	2.5	2.5	2.5	2.5	0.5	0.5
Aurofac, Lederle	1.2	1.2	1.0	1.0	1.0	1.0	0.25	0.25
Pasture intermittently [c]	501.2	501.2	501.2	501.0	501.0	501.0	100.25	100.25

[a] Purchased on the open market.

[b] This specimen (TCESOM–1, table 1, Pritchard *et al.*, 1956) produced aplastic anemia when fed to cattle.

[c] The pigs had excellent pasture from May 26 to Oct. 6, 1952. Some pasture was still available after Oct. 6, but alfalfa meal was added as a precautionary measure and to increase the bulk in the ration. The gilts were placed in concrete paved lots Feb. 15, 1953, and remained there until after they farrowed in April. They were placed on brome grass pasture when their litters were about one week of age and left there until June 16. They were returned to dry lot for the interval from June 16 to July 1 when the last of them were slaughtered.

Data on the reproductive performance of the pigs are summarized in table 6. Of the four gilts which were selected for breeding, for unknown reasons only two became pregnant in lot 2. At birth the average weight of the young in lot 2 was greater than in lot 1 in spite of the fact that the gilts in lot 2 produced a larger number of young per litter. The latter condition may account for the somewhat greater mortality of the young shortly after birth.

TABLE 2. SCHEDULE OF FEEDING VARIOUS RATIONS TO PIGS

Time interval	Ration fed to	
	Lot 1 (Hexane)	Lot 2 (TCESOM)
Parent generation		
20 May 1952–2 Aug. 1952	H_1	T_1
2 Aug. 1952–6 Oct. 1952	H_2	T_2
6 Oct. 1952–1 July 1953	H_3	T_3
F_1 generation		
Birth (2nd half April 1953–16 June 1953)	(available as creep feed with pasture)	
16 June 1953–12 Oct. 1953	H_1 (fed in dry lot)	T_1

TABLE 3. AVERAGE WEIGHTS OF ANIMALS OF PARENT GENERATION

Date	Ration fed			
	Lot 1 (Hexane)		Lot 2 (TCESOM)	
	Weight	No. of pigs	Weight	No. of pigs
	lb.		lb.	
May 20, 1952	26.8	6	26.8	6
Aug. 2	130.3	6	124.7	6
Oct. 6	231.7	6	219.1	6
Nov. 11	304.0	4[a]	292.0	4[a]
Feb. 3, 1953	414.0	4	387.0	4
4 days prior to farrowing	538.5 553.0[b]	4 2[b]	529.0	2[b]
Weaning, June 16	452.0	2	346.0	2
Loss per head from prefarrowing through lactation	101.0	2	183.0	2
July 1, 1953	442.0	2	367.0	2
Gain per head from June 16 to July 1, 1953	−10.0	2	+21.0	2

[a] Two pigs in each group slaughtered.
[b] Those that suckled their pigs for 8 weeks.

TABLE 4. NET GAIN OF ALL ANIMALS OF PARENT GENERATION

Dates	Ration			
	Lot 1		Lot 2	
	Hexane meal		TCESOM	
	lb.		lb.	
May 20–Aug. 2, 1952	621		587	
Aug. 2–Oct. 6	608		567	
Oct. 6–Mar. 31, 1953	1120		992	
Mar. 31 to farrowing	113		116	
Total	2462		2262	
Av. daily gain 5/20/52 to farrowing April 1953[a]	1.48		1.37	
Net weight loss from farrowing to slaughter	336	(4 sows)	324	(2 sows)
Total net gain from 5/20/52 to 7/1/53	2126		1938	
Av. daily gain from 5/20/52 to 7/1/53[a]	1.15		1.08	

[a] Calculated on pig day basis (1 pig×1 day=1 pig day).

TABLE 5. FEED CONSUMED DURING THE DIFFERENT PERIODS BY ALL ANIMALS OF PARENT GENERATION

Dates	Ration	
	Hexane meal	TCESOM
	lb.	lb.
May 20, 1952–Aug. 2	1975	1910
Aug. 2–Oct. 6	3031	3096
Oct. 6, 1952–Feb. 3, 1953	5511	4759
Feb. 3–July 1	4166	3379
Total	14683	13144
Creep feed for pigs	348	355
Grand total	15031	13499
Net gain of sows (table 4)	2126	1938
Total weight of pigs produced	565 (4 litters)[a]	500 (2 litters)
Total	2691	2438
Feed per 100 lb. net gain of all animals from 5/20/52 to 7/1/53	559	554

[a] Includes weaning weight of 2 litters raised to weaning and birth weight of 2 other litters.

TABLE 6. WEIGHT GAINS AND REPRODUCTIVE PERFORMANCE OF SWINE

Items compared	Lot 1 Fed hexane-extracted meal	Lot 2 Fed TCESOM
No. of gilts	4	4
Average weight 3 Feb. 1953, lb.	414	387
Average weight 2 days before farrowing, lb.	538	529
Average weight of non-pregnant gilts, lb.	...	468
No. of gilts not pregnant	0	2[a]
No. of gilts farrowed	4	2
Dates of farrowing	19 April–1 May	19 April–28 April
Total No. of live pigs farrowed	38	22
No. of stillbirths	0	2
Average No. of live pigs per gilt	9.5	11.0
Average birth weight of pigs, lb.	2.49	2.79
Vigor of pigs at birth		
Strong, %	63	68
Medium, %	5	14
Weak, %	32	18
Average No. of pigs weaned (56 days)[b]	7.0	7.5
Average weight at weaning[b]	38.3	35.4

[a] Slaughtered 21 April 1953.
[b] Includes only 2 litters from each lot.

Table 7 gives a summary of the growth performance of six F_1 generation pigs in each group. As in the case of their parents the pigs fed the TCESOM containing ration made somewhat slower daily gains but the efficiency of feed utilization was the same in both groups. Again the growth rate of the pigs was excellent.

Periodic biochemical and hematologic examinations were made of the blood of all pigs. These included analyses for glucose, blood urea, pantothenic acid of whole blood, hemoglobin as well as determinations of the clotting time, hematocrit, erythrocyte count, total and differential leukocyte count and the platelet count. Except as noted below all analyses and counts in both lots, in the parent and F_1 generation

TABLE 7. GROWTH AND FEED CONSUMPTION OF F_1 GENERATION PIGS

Items compared	Lot 1 Hexane	Lot 2 TCESOM
No. of pigs	6[a]	6[a]
Av. initial weight (16 June 1953), lb.	41.7	39.0
Av. final weight (at slaughter)[b] lb.	210.3	208.2
Av. daily gain, lb.	1.62	1.53
Av. daily feed consumption, lb.	6.0	5.6
Feed consumed per 100 lb. gain, lb.	368	366

[a] Each lot consisted of 3 pigs from each of two litters of dams fed rations containing hexane-extracted soybean oil meal (Lot 1) or TCESOM (Lot 2) since they were weaned.

[b] Pigs were removed for slaughter on an individual basis when they reached slaughter weights (range of weights 203–220 lb. in Lot 1; 201–222 in Lot 2) during the period of 23 September to 12 October 1953.

yielded results which fall into the range of normal values for swine of the age observed. These data will therefore not be given in detail. The exceptions noted are as follows: (1) three of the six pigs of the F_1 generation, born to gilts of lot 2 (fed TCESOM) showed, two weeks after birth, evidence of a mild anemia with hemoglobin concentrations of 7.8, 8.0 and 8.7 gm. per 100 ml. respectively and erythrocyte counts of 5.4, 4.6 and 4.6 million per cubic mm., respectively. This condition corrected itself without therapeutic measures. The corresponding average values recorded at the same time for the six F_1 generation pigs in lot 1 and for the three other pigs in lot 2 were in the range of 10 to 11 gm. of hemoglobin and over 6 million erythrocytes. (2) The total leukocyte count of the pigs in lot 2 (fed TCESOM) among the parent generation and particularly towards the end of the experiment in the F_1 generation was lower than observed in the corresponding groups fed the hexane-extracted soybean oil meal. As shown in table 8 the mean values of the leukocyte counts made during

four consecutive periods indicated that in the F_1 generation they were consistently lower in the pigs of lot 2 whereas the hemoglobin remained normal.

Daily observations of the pigs failed to reveal any abnormalities in either lot throughout the trial. At slaughter all carcasses were examined and in some instances specimens were taken for histologic examination. In two of the animals of the F_1 generation of lot 2 which had a temporary anemia as indicated above, there was evidence at slaughter of a chronic pericarditis. This condition frequently occurs in pigs afflicted with temporary anemia during the early post-natal period.

The experiments summarized above show clearly that swine are much more resistant than cattle to the toxic effects of the factor in TCESOM which produces aplastic anemia in cattle. In the evaluation of the results summarized in tables 3 to 8, two factors have to be taken into account:

TABLE 8. MEAN HEMATOLOGIC VALUES FOR F_1 GENERATION PIGS

Period (days)	Lot 1 (Hexane)			Lot 2 (TCESOM)		
	Hb[a]	Wbc[b]	% Neut.[c]	Hb[a]	Wbc[b]	% Neut.[c]
I (48)	11.3	19.3	26.7	10.3	15.7	28.0
II (28)	11.0	21.4	32.0	11.3	15.0	30.0
III (28)	11.4	18.1	21.0	11.8	11.6	17.0
IV (28)	12.0	17.1	25.0	11.6	10.9	24.0

[a] Hemoglobin, grams per 100 ml.
[b] Total leukocytes, thousands per mm^3.
[c] Percent neuterophiles.

(1). The two types of soybean oil meal, of necessity, did not originate from the same soybeans. In view of the different origin and age of the soybeans differences in nutritive value could be due to factors other than those associated with the solvent extraction of the beans.

(2) To simulate practical conditions the soybean oil meal was fed at the level of 10 to 20% of the concentrate ration which, in a relatively nonsusceptible species such as swine may not be high enough to reveal toxic effects, if any, of TCESOM.

It is not uncommon that partial nutritional deficiencies which cannot be detected during the growth period of an animal become accentuated under the stress of reproduction and lactation and that the offspring is adversely effected. It might be expected that a toxic compound in the maternal ration, otherwise undetected could reveal its presence during pregnancy, parturition and lactation of the dams and through the early

post-natal survival and growth of the offspring. If TCESOM had appreciable toxicity for pigs this might have become apparent during the reproductive phase of this experiment, in spite of the small numbers of animals involved. The only marked differences however between the two lots noted during the reproductive period were the apparent sterility of two of the four pigs fed TCESOM and the greater weight loss of the lactating dams fed the same ration. The offspring appeared to be normal. The moderate leukopenia observed in some pigs fed TCESOM might be indicative of slight toxicity but more extensive evidence would be required to make this observation conclusive.

It is apparent from the data that swine have a much smaller susceptibility, if any, to the toxic factor for cattle present in some specimens of TCESOM. Thus the F_1 generation pigs in lots 2 consumed in 100 days about 560 lb. of feed containing 20% of TCESOM or on the average about 1 lb. per day per 100 lb. of body weight. When young cattle were fed the same specimen of TCESOM at the same level of intake per 100 lb. of body weight they revealed symptoms of aplastic anemia in from 28 to 31 days and all five animals thus fed died in from 35 to 55 days (Pritchard *et al.* 1956).

Summary

A specimen of TCESOM of known, high toxicity to cattle was fed to swine at a level of 10 to 20% of the total ration throughout their period of growth and reproduction and to the offspring until they reached market weight. No decisive evidence of toxic effects was obtained. Swine are much more resistant than cattle to the toxic effects of the factor present in TCESOM which produces aplastic anemia in the bovine.

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EFFECT OF ANTIBACTERIAL AGENTS ON THE GROWTH OF SUCKLING PIGS [1]

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MANY workers have studied the value of various antibiotics when added to artificial milk diets for pigs weaned at a few days of age (Nesheim and Johnson, 1950) (Wahlstrom *et al.*, 1950) (Nesheim *et al.*, 1950) (Sheffy *et al.*, 1952) (Wahlstrom *et al.*, 1952) (Williams *et al.*, 1952) (Catron *et al.*, 1953). Schendel and Johnson (1952) included studies of arsanilic acid, seven surfactants, two sulfa drugs and six antibiotics in their experiments. From these various studies positive effects on growth of baby pigs have been noted, in one or more experiments, with chlortetracycline (Aureomycin), oxytetracycline (Terramycin), penicillin, streptomycin, chloromycetin, and arsenilic acid (p-aminophenylarsonic acid). Rimocidin, sulfathalidine, sulfisoxazole and the various surfactants used by Schendel and Johnson (1952) failed to produce a significant growth response.

Much less work has been done, apparently, to determine the value of these drugs when added to pig starters or creep feeds for pigs suckling the sow. In work at the Nebraska Station (Hanson, 1951), no consistent effect on growth was obtained from the feeding of streptomycin at levels of 1.8 or of 6.3 mg. per pound of creep feed. On the other hand Carpenter (1951) reported a significant increase in weaning weights from the addition of chlortetracycline to the creep ration. Oldfield and Hale (1952) reported increased weaning weights when suckling pigs were given intramuscular injections of penicillin in sesame oil and a lesser response when the same dosage (280 mg.) was given orally by capsule. Terrill *et al.* (1952) reported that antibiotics added to two different starters did not produce faster gains in suckling pigs fed on pasture. Lehrer *et al.* (1953) fed one capsule daily, containing 2.5 mg. chlortetracycline or oxytetracycline, to suckling pigs from 2 days to 4 weeks of age. In one experiment no significant response was obtained

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whereas in a second experiment the treated pigs made significantly greater gains up to 8 weeks of age.

A different approach was reported by Noland *et al.* (1952). The Arkansas workers reported increased weaning weights of pigs which had been implanted subcutaneously with bacitracin pellets at 2 days of age.

The present series of studies was begun in 1951 to obtain information on the value of several antibiotics when added to creep rations for suckling pigs. Later the series was expanded to measure the effect of bacitracin implants and to compare the value of chlortetracycline, procaine penicillin and arsanilic acid, when added to a creep ration. Part of the data was reported earlier (Hanson and Ferrin, 1952).

Procedure

Spring 1951

The pigs used in all trials were purebreds from the Station herd. They included 3 breeds, Chester White, Duroc and Poland China. Within each season, all pigs of a breed had the same sire.

In the first trial 16 litters, including 111 pigs, were divided into 4 groups of 4 sows and litters each, primarily on the basis of litter size and dam. The mean initial age of the pigs was approximately 5 weeks and they were fed for 23 days. In Trial 2, 10 litters including 72 pigs were allotted into 2 groups. They were started at about 3 weeks of age and fed for 7 weeks. In Trial 3, 10 litters with a total of 74 pigs were fed in two groups. They were slightly less than 3 weeks old intially and were fed for 5 weeks. Allotment in both Trials 2 and 3 was primarily on the basis of litter size and dam.

The dams (gilts) of the pigs in all trials had been fed the same ration throughout gestation and early lactation. During the experiment they were fed the mixture given in table 1, without a B_{12} supplement. The gilts had farrowed on concrete floors and remained on concrete with their pigs throughout the lactation period. In this series of trials the dams were hand fed twice daily in troughs, and no record was kept of the feed consumed. They were housed in a shed, but were fed on outside, paved runways. The creep area was in one corner of the outside feeding area. Automatic water supplies were provided. Anemia prevention in the pigs, prior to and during the experiment was attempted by providing the pigs with soil that had been stored through the winter in the barn. The basal creep mixtures fed are given in table 1. The creep mixture was fed in troughs and was given fresh daily. Antibiotics

were provided as feed supplements in Trial 1. The bacitracin and chlortetracycline supplements also contained vitamin B_{12}. The oxytetracycline supplement did not contain B_{12}. Crystalline procaine penicillin was used in Trials 2 and 3. At that time it was assumed that the sows' milk would provide adequate B_{12} and that the presence of B_{12} in two of the supplements fed was of no consequence.

The pigs were vaccinated for hog cholera with anti-cholera serum and virus about 2 weeks before weaning, in each trial.

TABLE 1. BASAL MIXTURES FED

Mixtures fed	Lactation ration for sows	Creep mixtures for pigs		
		Spring 1951	Spring 1952–1953	Fall 1952
Ingredients	lb.	lb.	lb.	lb.
Yellow corn, ground, No. 2	49.7	40.0	60.0	60.0
Oats, ground	25.0	....	....	....
Rolled oats, feed grade	....	40.0	30.0	25.0
Soybean oil meal[a]	7.0	9.5	4.5	4.5
Tankage (dry rendered, 60%)	7.0	10.0	5.0	....
Dried skim milk	....	....	....	10.0
Alfalfa hay (ground)	10.0	....	....	....
Mineral mixture (complex)	1.0	....	....	....
Salt (iodized)	0.3	0.5	0.5	0.5
Vitamin B_{12}[b]	5 mcg. per lb	....	5 mcg. per lb.	5 mcg. per lb.

[a] Expeller process in 1951; solvent process in 1952 and 1953.
[b] No B_{12} added to sow ration in 1951.

Spring 1952

It was observed in the 1951 experiments that the location of the creeps in the outside pens was not desirable. During periods of inclement weather the pigs preferred to stay indoors. Trough feeding of the dams seemed undesirable too, because the pigs attempted to compete with the sows for their feed rather than eating the creep ration provided for them. The feeding of soil to prevent anemia was also unsatisfactory. The soil had dried out during storage and was not well accepted by the pigs. Gross observation indicated that there were anemic pigs among those fed in 1951. Thus several important changes were made in the management procedures for 1952.

All of the dams were gilts that had been fed in two groups on one of two rations during gestation. The gestation rations (self-fed) differed in that one contained ground corn, oats, 25% alfalfa meal and supple-

ment, and the other contained ground corn, 45% alfalfa meal and supplement, but no oats. From the time the gilts were penned for farrowing until they were sorted for these trials all of them were fed the same lactation ration. During the experimental period all of the dams were self-fed the lactation ration given in table 1.

Anemia was prevented by swabbing the udder of each gilt once daily with a saturated "Copperas" solution (1 lb. crude $FeSO_4$ dissolved in ½ gallon water). Swabbing started when each litter was 3 or 4 days old and continued until the pigs were started in the experiment. Soil was provided in the creep pen for 2 weeks or more of the experimental period. The experimental pens were arranged indoors so that each group of sows could remain inside if they wished and an adjacent pen was used as a creep. Each group had access to an outside paved area on the south side of the building. Automatic water supplies were provided. As soon as sufficient numbers of sows and litters were available they were grouped for the tests. The groups were made up on the basis of breed, litter size, weight of pigs, and previous treatment of dams. Trial 4 included 2 lots of 5 litters each; Trial 5 included 4 lots of 4 litters each; Trial 6 included 2 lots with a total of 9 litters and Trial 7 included 2 lots of 4 litters each.

Crystalline vitamin B_{12} (in Mannitol) was included in all creep rations. See table 1. Crystalline procaine penicillin was used in Trial 4 and 20% procaine penicillin supplement was fed in Trials 5, 6 and 7. Vitamin B_{12} and the penicillin supplements were premixed with soybean meal before mixing with the other ingredients of the creep mixtures. The creep mixtures were prepared in batches of 200 lb. for each lot and thus were relatively fresh when fed. The pigs were vaccinated for cholera, as in the previous year, about 2 weeks before weaning.

Fall 1952

The pigs used in this experiment were those produced by gilts which had been fed on two levels of feed intake from a weight of 125 lb. (December) to farrowing (August). Each litter was treated as a separate experiment. About one-half of the pigs in each litter was implanted with one bacitracin pellet (1000 units) and the other pigs in the litter were not implanted and served as controls. The pigs in each litter were ear-marked and weighed within 24 hours after birth. In ear marking a system is used whereby each pig has a litter number in the left ear and an individual number in the right ear (i.e., 0 through 9). The pigs are sorted for sex and the gilts are marked first. Within

each sex the pigs are marked with no attention to the size or strength of the pig. To avoid confusion all even numbered pigs (0–2–4–6–8) were considered as controls and were not implanted. The odd numbered pigs (1–3–5–7–9) in each litter were implanted at 3 days of age. A few pigs were lost between birth and the third day, but no adjustment was made in the litter. For example, in one litter of nine there were 5 even numbered pigs and only 3 odd numbered pigs alive on the third day after birth. To avoid confusion only the 3 pigs were implanted. Any litter with an odd number of pigs automatically had one more pig in the control group, because each litter started with pig number 0. Thus there were a few more pigs in the control group than in the implanted group.

The pigs were farrowed in the barn but were placed on pasture at 4 to 7 days of age. All sows were self-fed the same ration. All pigs were creep-fed the mixture given in table 1. No antibiotic was fed either to the sows or to the pigs during the experiment. All pigs were vaccinated for cholera, with serum and virus, at about 6 weeks of age.

Spring 1953

Management in this experiment was similar to that followed in the Spring of 1952, given above. Two trials were completed. The first included 4 lots of 27 to 29 pigs per lot fed for 35 days. They were started at an average age of about 19 days. Trial 2 included 4 lots with 4 litters totalling 30 or 31 pigs per lot. They averaged about 22.5 days of age at the start and were fed for 28 days.

Vitamin B_{12} was supplied to lots 1, 3 and 4 in a feed supplement (Profactor-B, Lederle); vitamin B_{12} and chlortetracycline was supplied to lot 2 in a feed supplement (Aurofac). Pro-Pen "4" (Merck) was the source of procaine penicillin. Crystalline arsanilic acid (Abbott) was fed to lot 4. The basal creep mixture and the lactation ration for the sows are given in table 1.

Results and Discussion

Spring 1951

The results of the first experiment are given in table 2.

Bacitracin, at the level fed, did not improve the rate of gain of the suckling pigs, whereas chlortetracycline and oxytetracycline were effective. Perhaps the somewhat smaller initial weight of the bacitracin-fed pigs was a contributing factor. These pigs (lot 2) ate much less creep-feed than the other lots, for no apparent reason. The pigs were vaccinated for cholera 7 days after the test started. About one week later

TABLE 2. BACITRACIN, CHLORTETRACYCLINE AND OXYTETRACYCLINE AS SUPPLEMENTS TO A CREEP MIXTURE FOR SUCKLING PIGS, TRIAL 1, MARCH 26 TO APRIL 18, 1951

Lot No.	1	2	3	4
Ration supplement[a]	None	B_{12} Bacitracin	B_{12} Chlor-tetracycline	Oxy-tetracycline
No. of pigs	28	26	28	29
Mean age at start (days)	38.4	37.5	37.2	35.0
Av. initial weight, lb.	17.2	15.7	17.1	17.1
Av. final weight, lb.	25.8	24.4	31.7	28.8
Av. daily gain, lb.	0.37	0.37	0.64	0.51
Av. creep feed eaten per pig per day, lb.	0.47	0.31	0.47	0.45

[a] B_{12} fed at a level of 5 mcg. per lb. of feed for lots 2 and 3. The antibiotics were fed at a calculated rate of 5 mg. per lb. of feed.

scouring was severe in the control group and about one-half of the pigs in the bacitracin lot were scouring. None of the pigs fed either chlortetracycline or oxytetracycline showed any evidence of scours.

The results of Trials 2 and 3, given in table 3, show that procaine penicillin at the level fed did not stimulate the growth of the pigs. In Trial 3, the pigs fed penicillin ate very little creep feed. Considering the age of the pigs, the weaning weights were light in both trials.

There was a period of scouring for several days in all of the lots in Trials 2 and 3 preceding vaccination. The scouring started about 10 days after the pigs were placed in the test pens in each trial. Since the two trials were started two weeks apart, it appears that the scouring

TABLE 3. PROCAINE PENICILLIN AS A SUPPLEMENT TO A CREEP MIXTURE FOR SUCKLING PIGS, SPRING 1951

	Trial 2 April 24 to June 12		Trial 3 May 8 to June 12	
Lot No.	1	2	1	2
Ration supplement[a]	None	Penicillin	None	Penicillin
No. of pigs	35	37	37	37
Mean age at start (days)	23.5	22.4	20.4	19.8
No. days fed	49	49	35	35
Av. initial weight, lb.	10.4	9.9	8.7	8.6
Av. final weight, lb.	34.6	33.1	22.5	20.6
Av. daily gain, lb.	0.49	0.47	0.39	0.34
Av. creep feed eaten per pig per day, lb.	0.35	0.33	0.31	0.15

[a] Crystalline procaine penicillin added at a rate of 5 mg. per lb. of feed.

was associated with certain conditions pertaining to the pigs' development or environment, other than weather conditions. The pigs were eating very little creep feed during the time that scours occurred. Therefore, it would be unreasonable to expect that the antibiotic could be effective in the situation that prevailed. These negative results are included in this report in order to emphasize the fact (generally understood, but frequently overlooked) that management factors can easily negate ration treatments of young pigs. It is believed that many of these pigs were anemic. Furthermore it was found that the pigs did not like the creep arrangement and hence did not use it as they were expected to do.

Spring 1952

Because of the lack of significant results in the 1951 experiment with penicillin and because penicillin had given a significant increase in rate of gain when fed to weaned pigs in 1951, (Hanson *et al.*, 1955), the penicillin level was raised to 20 mg. per lb. of feed in the first trial in 1952. The results of this trial (Trial 4) and of Trial 5 are given in table 4.

The pigs used in Trial 4 were an excellent group of thrifty animals. It will be noted that the pigs in the control lot consumed 0.61 lb. of creep ration per pig daily and gained an average of 0.66 lb. per head daily. The pigs fed penicillin (lot 2) consumed an average of 0.93 lb. of creep ration daily and gained an average of 0.92 lb. per head daily. Covariance analysis (Snedecor, 1946) of initial weight and final weight, given in table 5, showed that the difference due to treatment was highly significant ($P<.01$). Because the dams of the pigs in lot 2 (Trial 4) also gained more weight than the control sows, the feed requirement per 100 lb. net gain of sows and pigs was 25% less for lot 2, the pigs whose creep ration contained 20 mg. of procaine penicillin per pound of feed.

In Trial 5 (table 4) three different levels (5, 10 and 20 mg. per lb.) of penicillin were fed in an attempt to determine an optimum level. The pigs in this experiment were weaned at 7 weeks of age so that subsequent experiments could be started before the pigs became too heavy. Consequently the weaning weights are lighter and feed consumption by the pigs is less than in Trial 4. Covariance analysis of adjusted initial and final weights (table 6) showed that the pigs fed 10 mg. penicillin per lb. of feed were significantly ($P<.05$) heavier than the controls and that the 5 and 20 mg. levels produced differences

that were significant at the 1% level. The two latter groups consumed much more creep feed daily than the controls or the pigs fed the 10 mg. level of penicillin. There was no apparent reason for the low level of feed intake by the pigs in lot 3. However, the low feed consumption

TABLE 4. THE EFFECT OF THE ADDITION OF VARIOUS LEVELS OF PROCAINE PENICILLIN TO A SIMPLE CREEP MIXTURE FOR SUCKLING PIGS, SPRING 1952

	Trial 4 March 10 to April 14		Trial 5 March 26 to April 23			
Lot No.	1	2	1	2	3	4
Penicillin per lb. feed	None	20 mg.	None	5 mg.	10 mg.	20 mg.
No. of litters	5	5	4	4	4	4
No. of pigs	34	33	30	29	30	30
No. days fed	35	35	28	28	28	28
Mean age of pigs at start (days)	21.9	22.2	22.1	21.6	20.8	21.9
Av. initial wt. of sows, lb.	382	396	367	378	396	356
Av. final wt. of sows, lb.	394	417	377	406	409	372
Av. gain per sow, lb.	12	21	10	28	13	16
Av. initial wt. per pig, lb.	11.8	12.0	10.8	11.0	11.0	10.9
Av. final wt. per pig, lb.	35.1	44.3**	27.3	33.5	30.2	31.2
Mean adjusted final weight, lb.[a]	.....	.....	27.48	33.30**	30.11*	31.31**
Av. daily gain per pig, lb.	0.66	0.92	0.59	0.80	0.69	0.73
Av. daily feed per sow and litter						
Sow ration, lb.	16.9	15.4	15.1	16.1	16.0	17.1
Creep ration, lb.	4.2	6.2	3.2	5.1	3.4	4.9
Total, lb.	21.1	21.6	18.3	21.2	19.4	22.0
Creep feed per pig per day, lb.	0.61	0.93	0.43	0.71	0.45	0.66
Feed per 100 lb. net gain of sows and pigs, lb.	432	322	385	311	344	365

[a] Adjusted according to average initial weights and regression (byx) = 2.06.
* $P<.05$; ** $P<.01$. See tables 5 and 6.

by the pigs is probably the reason why these pigs had significantly lower final weights than the pigs fed the 5 mg. level of penicillin.

There was no scouring at any time in Trial 4. In Trial 5 there was some trouble with scours. The incidence was highest in lot 1 and next highest in lot 3. On the basis of "pig days" (one pig observed scouring one day) there were 63 pig days of scouring in lot 1; 8 in lot 2; 42 in lot 3 and only 4 in lot 4. These values can be kept in proper perspective if it is noted that there were 840 pig days each in lots 1, 3 and 4 and 812 pig days in lot 2.

TABLE 5. COVARIANCE ANALYSIS OF INITIAL WEIGHT (X) AND FINAL WEIGHT (Y). TRIAL 4, SPRING 1952

Source of variation	DF	Sum of squares and products			Errors of estimate		
		X^2	XY	Y^2	Sum of squares	DF	M.S.
Total	66	282.5	639.4	4379.9			
Between litters	9	117.1	295.5	2749.7			
Between litters on same ration	8	116.8	275.3	1337.1			
Between rations	1	0.3	20.2	1412.6			
Within litters (error)	57	165.4	343.9	1630.2	915.2	56	16.34
Ration+error	58	165.7	364.1	3042.8	2242.8	57	
For testing significance of adjusted ration means					1327.6	1	1327.6**

** P<.01.

Trials 6 and 7 are summarized separately in table 7. It was planned to replicate Trial 5, but because of unexpected losses of several consecutive litters (diagnosed as uremia-toxemia) not enough litters were available at a given time. Thus, these trials were designed to find whether a low level (5 gm. per ton) of penicillin would be effective in promoting the growth of suckling pigs.

In Trial 6 the pigs fed penicillin did gain more rapidly than the controls, but their dams lost more weight than the control sows, even though the latter ate less feed daily. Thus it appears that the increased gains of the penicillin-fed pigs may be due to greater milk production by their dams. In this trial there were 17 pig days of scouring in lot 1 (out of a total of 1120 pig days) and no scouring in lot 2.

TABLE 6. COVARIANCE ANALYSIS OF INITIAL WEIGHT (X) AND FINAL WEIGHT (Y). TRIAL 5, SPRING 1952

Source of variation	DF	Sum of squares and products			Errors of estimate		
		X^2	XY	Y^2	Sum of squares	DF	M.S.
Total	118	568.0	1307.2	5887.6			
Between litters	15	350.2	857.6	3375.8			
Between litters on same ration	12	349.3	839.0	2780.0			
Between rations	3	0.9	18.6	595.8			
Within litters (error)	103	217.8	449.6	2511.8	1583.7	102	15.52
Ration+error	106	218.7	468.2	3107.6	2105.3	105	
For testing significance of adjusted ration means					521.6	3	173.87**

** P<.01.

Least Significant differences at 5% and 1%

N	29	30
29	2.07	2.06
	2.76	2.74
30		2.04
		2.71

In Trial 7 there was no appreciable difference between the feed consumption rate of the two lots of pigs nor in the daily rate of gain. In this trial the sows in lot 2 ate more feed than the control sows and consequently gained weight. Because of this the feed required per unit of net gain of sows and pigs was somewhat less for lot 2. There were

TABLE 7. THE EFFECT OF THE ADDITION OF A LOW LEVEL OF PROCAINE PENICILLIN TO A SIMPLE CREEP MIXTURE FOR SUCKLING PIGS, SPRING 1952

	Trial 6 April 14 to May 19		Trial 7 April 23 to June 3	
Lot No.	1	2	1	2
Penicillin per lb. feed	None	2.5 mg.	None	2.5 mg.
No. of litters	4	5	4	4
No. of pigs	32	38	28	28
No. days fed	35	35	41	41
Mean age of pigs at start (days)	21.2	22.8	16.9	15.7
Av. initial wet. of sows, lb.	374	396	360	343
Av. final wt. of sows, lb.	363	358	346	355
Av. gain per sow, lb.	−11	−38	−14	12
Av. initial wt. per pig, lb.	10.7	11.5	7.8	7.6
Av. final wt. per pig, lb.	24.3	28.2	29.0	29.2
Av. daily gain per pig, lb.	0.39	0.48	0.52	0.53
Av. daily feed per sow and litter				
Sow ration, lb.	10.2	10.9	12.5	13.7
Creep ration, lb.	2.9	3.4	2.3	2.5
Total, lb.	13.1	14.3	14.8	16.2
Creep feed per pig per day, lb.	0.36	0.45	0.33	0.36
Feed per 100 lb. net gain of sows and pigs, lb.	467	557	453	406

12 pig days of scouring in lot 1 and 35 pig days of scouring in lot 2 (out of a total of 1148 pig days per lot).

A covariance analysis (initial weight and final weight) of the combined data in Trials 6 and 7 showed no significant difference between the control pigs and the pigs fed 2.5 mg. penicillin per lb. of feed.

Fall 1952

The results of the bacitracin pellet implant study are summarized in table 8.

The pigs that were implanted weighed 0.15 lb. more per pig at birth and 0.8 lb. more per pig at 55 days of age. According to Vestal (1936)

birth weights have considerable effect on weaning weights of pigs. His data show that 0.25 lb. per pig at birth is equivalent to 1.26 to 1.87 lb. at 8 weeks. The greater difference is found between pigs of light weights and the lesser difference between pigs in the 2.75 to 3.0 lb. weight bracket or heavier. Thus, the slight difference in weaning weight of the Group 2 pigs is completely accounted for by their advantage in birth weight. The fact that the treated animals were heavier at birth was strictly fortuitous because the pigs were selected for treatment without regard to birth weight (see procedure).

TABLE 8. THE EFFECT OF SUBCUTANEOUSLY IMPLANTED BACITRACIN PELLETS ON THE GROWTH OF SUCKLING PIGS, FALL 1952

Group	1	2
Treatment	None	Bacitracin[a]
No. of pigs at start (3 days)	111	96
No. of pigs at weaning	107	92
Av. age at weaning (days)	55	55
Av. birth weight, lb.	2.70	2.85
Av. weaning weight, lb.	28.8	29.6
Incidence of scours	zero	zero

[a] One bacitracin pellet of 1000 units was implanted beneath the skin of the pig at the base of one ear.

Weather conditions were nearly ideal for fall pigs in 1952. It was a dry fall (0.45 inch of rain in early September and about 0.10 inch in October) and most days were sunny and mild. It was concluded that, under the conditions imposed, bacitracin pellet implants did not increase the growth or survival of the pigs. Neither did the treatment have any harmful effect on the pigs.

These data disagree with the results reported by Noland *et al.* (1952). Lasley *et al.* (1953) also obtained results different from those in the present study. They reported highly significant increases in gains of pigs implanted with bacitracin pellets at 2 days of age, but not in pigs implanted at 2 weeks. In a second experiment at the Missouri Station no effect was obtained with implants of either bacitracin or a combination of penicillin and bacitracin. Lee (1953) has reported an increase of almost 22% in growth, up to 8 weeks, of pigs implanted with a bacitracin pellet.

On the other hand, Clawson *et al.* (1953) reported no effect on gains of pigs up to 8 weeks from antibiotic pellet implants. The antibiotics tested in their study included chlortetracycline, penicillin, bacitracin,

penicillin-bacitracin, chlortetracycline-bacitracin and chlortetracycline-penicillin. Likewise, Perry *et al.* (1953) reported negative results with bacitracin; Terrill *et al.* (1954) reported no effect from pellets of bacitracin, procaine penicillin G, chlortetracycline·HCl and chlortetracycline·Ca; and Wallace *et al.* (1954) obtained no significant effect on 4-week weights, 8-week weights or survival rate from the implantation of either bacitracin or chlortetracycline·HCl pellets.

Spring 1953

The 1951 and 1952 studies had demonstrated that chlortetracycline, oxytetracycline, or penicillin additions to a creep ration could increase the growth rate of pigs up to 8 weeks. Other studies at this Station (Hanson *et al.*, 1955a) indicated that arsanilic acid might also be an effective addition to a creep ration. Therefore, this experiment was designed to compare directly the value of chlortetracycline, penicillin and arsanilic acid. The linear model of analysis of variance was then assumed. Two trials were completed and the data are combined and summarized in table 9.

Covariance analysis (Snedecor, 1946) was made for each trial and for the combined data.

In the first trial the pigs were fed for 35 days. The adjusted mean final weights were 32.2, 36.5, 37.3 and 38.1 lb. for lots 1, 2, 3 and 4, respectively. Least significant differences at the 1% level were 3.45 to 3.48 lb. for the various lots. Thus each of the antibiotics and arsanilic acid significantly improved the growth of the pigs, but there was no significant difference between them. A similar analysis of the second trial, in which the pigs were fed for only 28 days, revealed no statistically significant effect on growth rate from the addition of any of the drugs. The adjusted final weights in this trial were 28.2, 29.8, 29.0 and 29.0 lb. for lots 1, 2, 3 and 4, respectively. Analysis of the combined data showed that the differences between trials and their interaction with rations were significant. The significant interaction was not unexpected in view of the difference in the length of the trials and the common observation that the last week before weaning (at 8 weeks) is the week of greatest feed consumption and live weight gain by the pigs. Studies dealing with the mode of action of antibiotics help to explain why one should not necessarily expect to obtain the same response each time such a drug is fed. The combined analysis of both trials given in table 10, does show that there is a significant difference ($P<.05$) between the rations. It is concluded that under the conditions of this experiment chlortetracycline,

procaine penicillin and arsanilic acid were equally effective in promoting live weight gains.

The supplemented pigs gained more weight because they ate more of the creep feed than the control pigs (table 9). The average feed consumption by the sows was highest for lot 2 and these sows gained

TABLE 9. CHLORTETRACYCLINE, PROCAINE PENICILLIN AND ARSANILIC ACID AS SUPPLEMENTS TO A CREEP RATION FOR SUCKLING PIGS, (THE DATA GIVEN ARE THE MEANS OF 2 TRIALS, SPRING 1953)

Lot No.	1	2	3	4
Treatment, amount per lb. feed	None	Chlortetracycline, 5 mg.	Procaine penicillin, 5 mg.	Arsanilic acid, 30 mg.
No. of litters	8	8	8	8
No. of pigs	59	58	58	59
No. of days fed[a]	31.5	31.5	31.5	31.5
Av. initial age (days)	19.5	20.2	22.1	21.7
Av. initial wt. of sows, lb.	386	402	381	381
Av. final wt. of sows, lb.	390	418	384	385
Av. gain per sow, lb.	4	16	3	4
Av. initial wt. per pig, lb.	10.0	10.2	10.3	10.2
Av. final wt. per pig, lb.	29.8	33.1	33.2	33.4
Mean adjusted final wt., lb.	30.2	33.0*	32.9*	33.3*
Av. daily gain per pig, lb.	0.63	0.73	0.73	0.74
Av. daily feed per sow and litter				
Sow ration, lb.	14.1	15.8	13.4	14.7
Creep ration, lb.	3.3	4.1	4.5	4.1
Total, lb.	17.4	19.9	17.9	18.8
Creep feed per pig per day, lb.	0.45	0.57	0.62	0.55
Feed per 100 lb. net gain of sows and pigs, lb.	364	343	332	336

[a] Length of feeding period was 35 days in the first and 28 days in the second trial.
* $P<.05$, see table 10.

the most weight during the experimental period. The sows in lot 3 ate the least feed, but this was compensated in part by the increased consumption of creep feed by their pigs. Overall there was a substantial saving in feed per unit of net gain of sows and pigs of the supplemented lots as compared with the controls. This amounted to approximately 6% to 9% for the various lots.

There was some scouring of the pigs in Trial 1. Scours first appeared in a few pigs in lot 4 about 10 days after the trial started. Within a few days there was some scouring in all lots. The scours period lasted for

about one week and had no apparent serious effect on the pigs. There were 35 pig days of scours in lot 1; 21 in lot 2; 33 in lot 3; and 67 in lot 4. (There was a total of 945 to 1015 pig days per lot in this trial). It is believed that a weather change precipitated the scouring. There was no scouring in trial 2.

Summary

One hundred and eleven litters, including 803 pigs, were used to study the values of chlortetracycline, bacitracin, penicillin, oxytetracycline and arsanilic acid as supplements to a simple creep mixture for suckling pigs. Most of the experiments included the period from 3 weeks to 8 weeks (age) of the pig's life. In addition, 207 fall pigs were used to study the value of bacitracin pellets (1000 units), implanted at 3 days of age into some pigs of each litter so that each litter served as its own control.

TABLE 10. COVARIANCE ANALYSIS OF COMBINED GROWTH DATA FROM 2 TRIALS, SPRING 1953

Source of variation	Sum of squares and products				Errors of estimate		
	DF	X^2	XY	Y^2	Sum of squares	DF	M.S.
Total	233	1550.06	3536.54	15721.7			
Rations	3	2.99	36.88	531.1			
Between trials (length of period)	1	0.97	52.20	2806.2			
Ration × trial	3	15.64	54.20	420.0			
Error	226	1530.46	3393.25	11964.4	7441.1	225	33.07
For test of significance of adjusted ration means					231.7	2	115.85*
For test of significance of adj. (ration × trial) means					246.0	2	123.00*

X=initial weight, Y=adjusted final weight. * $P<.05$.

Oxytetracycline and bacitracin were used only in a single experiment at levels of 5 mg. per lb. of creep ration. The former was quite effective in increasing the gains of the pigs and in preventing scours in the pigs following vaccination for cholera. Bacitracin was only partially effective in controlling scours and did not affect the rate of gain of the pigs. For reasons discussed the data obtained with bacitracin in the creep ration are considered inconclusive.

Procaine penicillin was without effect on the pigs to which it was fed in the first experiment. These negative results are attributed to faulty management techniques. In a series of later experiments procaine penicillin at levels of 5, 10 or 20 mg. per lb. of feed increased live weight gains of pigs significantly and reduced the feed requirement per unit

of net gain of the sows and their pigs. Penicillin was ineffective at a level of 2.5 mg. per lb. of feed.

In a final experiment in this series, chlortetracycline and procaine penicillin at levels of 5 mg. per lb. of feed, and arsanilic acid at a level of 30 mg. per lb. of feed produced statistically significant increases in weaning weights of the pigs in one trial. In a second trial effects on weaning weights were not significant. There was no significant difference between responses to the three drugs. As an average for both trials the antibacterial agents reduced the feed required per unit of net gain of sows and pigs 6 to 9%.

Under the conditions of the experiment the subcutaneous implantation of bacitracin pellets did not affect weaning weights or survival of the pigs to 8 weeks of age.

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PROTEIN LEVELS FOR PIGS AS STUDIED BY NITROGEN BALANCE

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IN recent years at Illinois, Iowa, and other experiment stations protein studies with various rations have been conducted with swine to determine the minimum protein requirement for growth. In those studies several methods of feeding and several criteria have been used, and the general conclusion reached has been that the minimum protein requirements are lower than was formerly believed.

Market preference for lean pork has stimulated development of a "lean type hog," and it has been a matter of concern whether such factors as the addition of antibiotics or vitamins, or the reduction of the protein allowances, has affected the proportion of lean in the carcass.

The same well-fortified practical diets that were used in growth studies reported previously (Lassiter *et al.*, 1955) were used to study the minimum protein requirement of pigs, using maximum nitrogen retention as the criterion. A secondary objective was to compare the precision of the results of 3-, 5-, and 7-day collection periods.

Experimental

A pilot test was conducted with one quartet of pigs to gain information for planning the nitrogen retention study.

For the study it was desired to use four quartets of pigs of approximately 50 lb. liveweight, and four quartets of pigs of approximately 150 lb. liveweight, but some variation from these desired weights was necessary because of practical considerations.

Small Pigs

One quartet of small pigs consisted of littermate Hampshire barrows, weighing 43 or 44 lb. at the beginning of the preliminary period. A second quartet consisted of very uniform Hampshire pigs weighing 49 or 50 lb., of similar breeding but not littermates, nor were they of one

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sex, being three gilts and one barrow. A third quartet consisted of very uniform Yorkshire x Hampshire pigs weighing 37 to 40 lb., of similar breeding but again not littermates, and again there were three gilts and one barrow. A fourth quartet consisted of uniform Yorkshire littermate barrows, weighing 42 to 46 lb. Each quartet was randomly allotted to protein levels and to the four 4′ x 6′ swine metabolism crates used.

TABLE 1. COMPOSITION OF DIETS FED ON NITROGEN RETENTION STUDY

Calculated protein, %	10	14	18	22
Protein (by analysis), %				
Small pigs[a]	10.1	13.9	17.9	22.4
Large pigs	10.1	14.1	18.3	21.9
Ingredients:				
Ground yellow corn (8.21% protein)	91.6	80.2	68.7	57.2
Protein supplement (43.05% protein)[b]	5.8	17.2	28.7	40.2
Mineral mixture No. 3[c]	2.0	2.0	2.0	2.0
Vitamin A & D oil (3000 A, 600 D)	0.5	0.5	0.5	0.5
Antibiotic supplement[d]	0.1	0.1	0.1	0.1
Vitamin mixture No. 36[e]	+	+	+	+

[a] Composite samples were not analyzed after the first quartet in either the large or small pig tests.

[b] Consisted of 50% solvent soybean oil meal, 15% dehydrated alfalfa meal, 15% meat and bone scrap (50% protein), 15% menhaden fish meal and 5% condensed menhaden fish solubles.

[c] Percentage composition of mineral mixture No. 3 was as follows: 40.0 ground limestone, 31.2 steamed bone meal, 25.0 iodized NaCl, 2.0 $MgCO_3$, 1.0 $FeSO_4 \cdot H_2O$, 0.56 $MnSO_4 \cdot H_2O$, 0.10 $CoCl \cdot 6H_2O$, 0.1 $CuSO_4$, 0.04 $ZnCO_3$.

[d] Guaranteed to supply 2 gm. procaine penicillin and 3 mg. vitamin B_{12} (L. L. elution assay) per pound.

[e] Additions per pound of ration were: 1.4 mg. riboflavin, 5.0 mg. calcium pantothenate, 5.0 mg. niacin, and 1.0 gm. choline chloride powder (25% choline chloride).

The composition of the rations is given in table 1. Since the pilot test had indicated that nitrogen retention increased up to 16% protein, it was decided to use protein levels of 10, 14, 18 and 22% in an attempt to embrace the level that would give maximum retention. Other than this difference in protein level, the rations were the same as those in the drylot growth test previously reported.

In all quartets the equalized feeding technique was used. Each pig was fed 2 lb. of feed per day divided equally into a morning and an evening feed. Each feed was made into a slop as an aid to consumption and to reduce wastage.

A 10-day preliminary period on the test diets, in the collection crates, preceded the collection periods. Each collection period was divided into a 3-day period and two successive additional 2-day periods. To identify the collection periods, ferric oxide was used as the feces marker at 0.5%

of the daily feed intake. The ferric oxide was mixed with one-tenth of the feed for the particular feeding and wet down. After this was cleaned up, the remainder of the feeding was fed.

The urine was collected in 4000 ml. brown jars in which 100 ml. of 6 to 8% acetic acid had been placed. After collection, the urine was strained through a 100-mesh wire strainer, diluted to a known volume and aliquoted for the nitrogen determination.

The feces were acidified with acetic acid, dried in a forced hot-air oven, allowed to come to equilibrium with the air moisture, weighed, ground in a knife mill (Wiley mill) to pass a 40-mesh screen, and aliquots were taken for the Kjeldahl analysis. The feed, feces, and urine were analyzed for nitrogen by the Kjeldahl-Wilfarth-Gunning process.

Large Pigs

One quartet of large pigs consisted of Yorkshire barrows of the same age and of similar breeding, weighing 149 or 150 lb. at the beginning of the preliminary period. A second quartet, consisting of Hampshire barrows weighing 152 to 162 lb., was purchased, each pig being immunized against cholera and erysipelas before being allotted to the test. A third quartet, consisting of crossbred pigs, was used to test the adaptability of sheep metabolism crates for swine, and so was kept in the crates for 10 days before beginning the preliminary period. At the time the pigs were placed in the crates, they weighed 114 to 132 lb. A fourth quartet, also crossbred pigs from the swine farm, weighed 152 to 166 lb. when started on the preliminary period.

The rations and the methods of feeding were the same as used with the small pigs. Each pig was fed 4.5 lb. of feed per day, divided equally into a morning and an evening feed.

As with the small pigs, the preliminary period consisted of 10 days on the ration used in the test before the collections were started. Except for the fourth quartet, the pigs were in the collection crates during the preliminary period. Because of space limitations, the fourth quartet was in individual wire-bottomed feeding cages during the preliminary period and was changed to the collection crates at the beginning of the collection period.

Collections from the first two quartets were made in the same crates used by the small pigs. Collections from the last two quartets were made in sheep metabolism crates. The inside dimensions of these crates were 33″ high, 48″ long, and 26″ wide at their widest adjustment, the metal feed box being inside this space.

As with the small pigs, the collection period consisted of 3 days, plus two successive 2-day periods, enabling the 3-, 5-, and 7-day comparisons to be made. Ferric oxide was used as the feces marker in the same manner and at the same rate as with the small pigs. The urine from each pig was collected daily in 3-gallon galvanized buckets in which 100 ml. of 12 to 14% acetic acid had been placed to prevent loss of nitrogen. The urine was then strained, diluted and aliquoted for the nitrogen de-

TABLE 2. AVERAGE APPARENT DIGESTIBILITY VALUES, AVERAGE CALCULATED BIOLOGICAL VALUES, AND AVERAGE DAILY NITROGEN RETENTION, SMALL PIGS

Protein level	Average apparent digestibility values	Average calculated biological values[a]	Average daily nitrogen retention
%	%	%	gm.
	First three days		
10	80.71	62.78	5.52
14	80.38	63.33	8.87
18	81.04	64.97	12.36
22	81.28	54.93	12.36
	Next two days		
10	80.92	58.35	5.31
14	80.55	64.18	9.01
18	84.16	65.05	13.00
22	81.33	52.15	11.58
	Next two days		
10	80.05	58.24	5.20
14	82.07	63.92	9.19
18	80.69	61.48	11.49
22	82.37	52.10	11.71

[a] Calculated by the Mitchell Method. J. Biol. Chem. 58:873, 1924; and J. Nutr. 11:257, 1936. Metabolic fecal nitrogen was computed on the basis of 1.18 gm. nitrogen of body origin for each 1000 gm. of dry matter consumed. (R. M. Forbes, personal communication). Endogenous nitrogen was computed by the formula $N=146W^{0.72}$ where N is in milligrams and W is in kilograms, as given by Brody, S. *et al.* Missouri Agr. Exp. Sta. Res. Bul. 220: 1934.

termination. In each quartet the feces were handled in the same manner as in the small pig collections. The nitrogen determinations were conducted in the same manner as with the small pigs.

Results

Small Pigs

The average daily nitrogen retention is shown in table 2. A linear regression on protein percentage accounts for most of the variance. However, by fitting a straight line through the two lower points and a

horizontal line through the two higher points, a somewhat closer fit resulted, suggesting that maximum retention was reached at a protein level somewhat less than 18%. An interesting observation was that, although the pigs on the higher levels of protein excreted a greater quantity of feces (dry weight) and these feces had a higher moisture content, the protein levels did not appear to affect the digestibility of the protein. On the other hand, except for one pig, the calculated biological values were less at 22% protein than at the lower levels, so that the decreased retention at that level might be caused by decreased utilization.

After a 10-day preliminary period, a 7-day collection period does not increase the precision over a 3-day collection period enough to warrant much increase in the cost of collection by increased time of operator, cost of feed, prolonged use of equipment, etc. The difference in precision, as shown by analysis of variance, was that 19 pigs on 7-day collection would yield the same error as 20 pigs on 5-day collection or 25 pigs on 3-day collection. It should be understood that the comparison of 3-day to 5-day to 7-day collection periods reported here is not exactly what it seems. In particular, the so-called 5-day and 7-day collections are really combinations of two or three shorter collections, which were analyzed separately. This means that the chemical errors are smaller, by being an average of two or three independent errors, in the 5-day and 7-day average daily retention figures, than would have been the case if 5-day collection meant a single collection representing a 5-day period with one sample taken for analysis, and similarly for the 7-day period. It is perhaps conceivable that combining shorter collections does not have the effect of reducing *all* errors relative to what they would have been for a single collection. It is our opinion that the comparison reported here is close to the truth but probably makes 5-day and 7-day collections appear better than they should relative to 3-day collections.

Large Pigs

The average daily nitrogen retention is shown in table 3. The data suggest that protein levels affect nitrogen retention, retention at the 10% level appearing less than at the higher levels, but statistical analysis showed that the differences are not significant. At the highest protein level, even after a 10-day preliminary period, it appeared that the average daily nitrogen retention was decreasing as the collection period progressed. If there is any real difference between protein levels, it would appear to be in utilization rather than in digestibility. Except for one

pig, the calculated biological values were less at the two higher levels than at the two lower levels, and except for one pig, the calculated biological values were less at the 22% than at the 18% level. On the other hand, the digestibility varied more between individual pigs on the same protein level than between protein levels.

TABLE 3. AVERAGE APPARENT DIGESTIBILITY VALUES, AVERAGE CALCULATED BIOLOGICAL VALUES, AND AVERAGE DAILY NITROGEN RETENTION, LARGE PIGS

Protein level	Average apparent digestibility values	Average calculated biological values[a]	Average daily nitrogen retention
%	%	%	gm.
	First three days		
10	80.04	53.47	10.26
14	85.05	52.10	16.39
18	86.24	44.74	18.76
22	83.61	37.25	17.66
	Next two days		
10	88.57	58.46	13.45
14	88.52	49.63	16.21
18	84.62	44.22	18.04
22	83.28	32.70	14.72
	Next two days		
10	82.54	51.71	10.14
14	86.79	50.08	15.96
18	85.78	43.41	17.81
22	82.06	29.83	12.70

[a] Calculated in the same manner as described in table 2.

Discussion

Although recent growth experiments had indicated a lower level of protein needed for maximum growth than was formerly recommended, current market demand for a leaner hog raised the question of whether the reduced protein allowances, or other factors like the addition of antibiotics or of vitamins, influence the nitrogen stored and the lean content of the carcass.

The results with the small pigs in this trial indicate that nitrogen retention increases to about the 18% protein level. With the large pigs the data suggest that protein levels affect nitrogen retention, but the differences are not statistically significant. In a previously reported growth test (Lassiter *et al.*, 1955) on the same diet, when the same protein level was fed from weaning to 100 lb., the minimum protein for

maximum growth appeared to be 14 to 16%; when fed from weaning to 200 lb. it appeared to be 12 to 14%. Thus it appears that protein levels which give maximum growth rates may be lower than the levels which give maximum nitrogen retention.

Mitchell (1939) obtained higher protein requirements in balance experiments than in growth experiments and concluded that equal gains in weight of growing pigs may contain different amounts of protein in response to different levels of protein in the rations.

That any study of the effect of protein levels on nitrogen retention needs to be closely correlated to the other factors in the diet is indicated by several studies. Brush *et al.* (1947) observed that deficiencies of different amino acids may exert different effects on nitrogen balance. They found that choline, cystine, and all of the essential amino acids *except* phenylalanine, valine and tryptophan, exerted some action in sparing body nitrogen. Robschiet-Robbins *et al.* (1947) showed that methionine, threonine, phenylalanine and tryptophan, when singly eliminated from a complete amino acid mixture effected a sharp rise in urinary nitrogen. This loss was corrected when any one of the individual amino acids was replaced in the mixture. However, they reported that histidine, lysine and valine had only a moderate influence upon urinary nitrogen balance, and that leucine, isoleucine and arginine had little or no effect upon urinary nitrogen balance.

In interpreting the results of nitrogen balance experiments there appear to be several other variables needing careful evaluation. Goldsmith, *et al.* (1952) summarized these as the following: the total daily caloric intake; the distribution of non-protein calories in the diet; the relation of total dietary protein to the caloric value of the ration; the time factor in feeding; the distribution of animal protein over the day's meals; the general physiologic state of the animal, and the status of body protein stores. Bell and Loosli (1951) showed that increases in weight caused marked decreases in the efficiency of nitrogen utilization for growth in pigs. Perlman and Cassidy (1953) showed that hormonal activity affects nitrogen retention. Effects of vitamins on nitrogen retention have also been shown (Brown and Morgan, 1948; Cheng and Thomas, 1952).

Variables such as these emphasize the importance of careful interpretation of experiments using nitrogen retention as a criterion. Indeed, they indicate that different results not only might occur in different experiments, but that different results should be expected unless all these factors are equal because the same protein level might give several levels of nitrogen retention by varying the factors.

The results of the study of the length of collection periods are of interest. A long collection period appears to offer little advantage following a preliminary period of sufficient length. In this study there appeared to be only a little advantage in the case of the small pigs and even less advantage in the case of the large pigs. However, with the large pigs some error was undoubtedly caused by difficulty in separating the collection periods on the lower protein levels.

Summary

Using well-fortified practical diets, a study was made of the minimum protein intake of pigs for maximum nitrogen retention. The precision of 3-, 5-, and 7-day collection periods was also compared.

With 50-lb. pigs, nitrogen retention increased to about the 18% protein level. With 150-lb. pigs, the data suggest that crude protein levels from 10 to 22% affect nitrogen retention, but the differences were not statistically significant.

After a 10-day preliminary period, a 7-day collection period offered only a little advantage over a 3-day collection period in the case of 50-lb pigs, and even less advantage with 150-lb. pigs.

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THE RELATIONSHIP BETWEEN THE BLOOD PLASMA VITAMIN A LEVELS OF SOWS AND OF THEIR SUCKLING PIGS [1]

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THE amount of vitamin A in the liver and blood plasma of newborn pigs is affected by the vitamin A content of the prepartum ration of the sow, (Thomas *et al.*, 1947; Whiting *et al.*, 1949). Dann (1933) has indicated that the amount of vitamin A in the liver of the newborn calf, however, is small compared to the quantity obtained from the colostrum of its dam. As colostral vitamin A levels are high in the sow (Bowland *et al.*, 1949, 1951) it is probable that the same relative relationship suggested for the calf would apply to the newborn pig. It has been reported by several groups of workers (Heidebrecht *et al.*, 1951; Thomas *et al.*, 1947; Wiseman *et al.*, 1949), that the colostral vitamin A levels are influenced by the dams' ration. If the level of vitamin A in the blood plasma of a suckling animal is governed largely by the amount of vitamin A which it ingests via the colostrum and milk, and if the total amount of vitamin A secreted in the milk, irrespective of volume, is governed by the vitamin A intake which will influence blood plasma level of the lactating animal; then it would appear possible that a relationship might exist between the blood plasma vitamin A levels of a nursing dam and her young. Such a relationship is suggested by the summarized data of a number of workers including Eaton *et al.* (1949), and Parrish *et al.* (1951). It was to determine whether such a relationship does exist in swine that the following experiment was conducted.

Experimental

During 1954, 16 Yorkshire sows, along with one male and one female pig from each of their litters, were selected at random for vitamin A studies. The sows were placed in one of four groups, according to the season in which they were farrowed, January, February-March, July, and September.

Sows were fed a 15% protein ration based on oats and barley in a

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TABLE 1. MEAN LEVELS OF VITAMIN A IN THE BLOOD PLASMA OF SOWS [a] (mcg. per 100 ml.)

Farrowing Season	Days after farrowing						
	7	14	21	28	35	42	49
January	35.2			22.2			22.5
February–March	28.6	38.0		37.2			39.8
July	49.2	41.5	36.1	33.9	47.2	41.5	41.0
September	49.8	39.9	35.8	40.9	48.3	35.2	36.3

[a] There were four sows in each farrowing period.

2:1 ratio with supplemental ground limestone and iodized salt. In the case of sows farrowing in the spring, 10% dehydrated alfalfa meal was fed during pregnancy when no pasture was available. During lactation the sows' ration was fortified with approximately 1,250,000 I.U. of vitamin A per ton.

A sample of approximately 15 ml. of blood was drawn from the anterior vena cava of each animal at weekly or less frequent intervals as indicated by tables 1 and 2, the method devised by Carle and Dewhirst (1942) being used. The oxalated blood was centrifuged and the plasma analyzed for vitamin A by the method of Kimble (1939).

Results

The mean values obtained for the vitamin A content of sows' blood plasma are reported in table 1, and those for their piglets are reported in table 2. As an analysis of variance indicated that there was no difference between the plasma vitamin A levels of the male and female piglets, the data for both sexes were pooled.

The raw data were subjected to a modified analysis of covariance as indicated in table 3 (Snedecor, 1946). As complete data were available for only the first, fourth and seventh weeks after farrowing, these alone were used in the analysis.

The first part of the analysis consisted of obtaining the regression

TABLE 2. MEAN LEVELS OF VITAMIN A IN BLOOD PLASMA OF YOUNG PIGS [a] (mcg. per 100 ml.)

Season born	Age in days						
	7	14	21	28	35	42	49
January	22.1			15.6			16.8
February–March	24.9	33.4		32.5			28.1
July	46.5	40.5	37.9	28.2	30.4	23.6	27.4
September	35.9	35.0	26.2	30.3	34.6	27.9	28.0

[a] Each mean is from eight pigs.

and correlation data (table 3). The next step was to determine whether the relationship between the plasma vitamin A levels of sows and their piglets held over the preweaning period; this was done by testing for the difference between the regression coefficients calculated for each of the three sampling dates. The difference between these regression coefficients was found to approach the 5% level of significance, ($F=2.80$ and F for P ($F \geqq F_{.05}$)$=3.10$) indicating that the relationship found in one or more periods differed from the other(s). The relationship found in the seventh week after farrowing seemed to be quite different from that found in either the first or fourth weeks, indicating that the influence of the sow on the plasma vitamin A level of her pigs had apparently decreased by the seventh week. Since repeated samples from the same group of sows and piglets were taken in each period these data cannot be assumed to be independent. However, if any bias occurs in the regression coefficients for each period, it should be in the direction of similarity and lend support to the conclusion that differences in the regression coefficients are real.

As probable differences in the correlation and regression coefficients for the first, fourth and seventh weeks were indicated, regression (prediction) equations were calculated separately for each of the three sampling periods as well as a general regression equation from the pooled data. These data appear in table 4. In the derived equation, Y is the level of vitamin A in the blood plasma of a young pig; $\bar{y}$ is the mean value for all young pigs measured in this experiment; $\dot{Y}$ is the predicted level of vitamin A in the blood plasma of the young pigs; X is the level of vitamin A in the blood plasma of the dam of the young pigs for which the prediction is to be made; $\bar{x}$ is the mean value for all the sows measured in this experiment; b_{yx} is the regression coefficient, or the change in Y for unit change in X.

$$\dot{Y}-\bar{y}=b_{yx}(X-\bar{x})$$
$$\dot{Y}=b_{yx}X-b_{yx}\bar{x}+\bar{y}$$
$$\dot{Y}=C+b_{yx}X$$

where $C=\bar{y}-b_{yx}\bar{x}$

Table 4 gives the values resulting from the inclusion of the raw data from this experiment into the foregoing equation.

Discussion

The results shown in tables 3 and 4, indicate that the vitamin A level of the blood plasma of suckling pigs is dependent upon the level of vitamin A in the plasma of their dams. It has been shown by Lewis

TABLE 3. REGRESSION AND CORRELATION DATA FROM PLASMA VITAMIN A STUDY [a]

Week	d.f.	Sums of squares and products			Correlation coefficient	Regression coefficient	Errors of estimate	
		SS_x	S_{xy}	SS_y	r_{xy}	b_{yx}	Sums of squares	d.f.
1	31	8199.99	4990.66	6383.33	0.690	0.609	3345.93	30
4	31	4815.39	2276.01	2410.24	0.668	0.473	1200.24	30
7	31	5384.95	1838.12	2028.28	0.556	0.341	1400.85	30
Sum	93	18400.33	9104.79	10821.85			5947.02	90
Total	95	19377.69	10002.80	11765.80				
Weeks	2	977.37	898.01	943.95				
Within weeks	93	18400.32	9104.79	10821.85	0.645	0.495	6316.65	92

[a] X is the level of vitamin A in the blood plasma of the dam of the young pigs for which the prediction is to be made and Y is the level of vitamin A in the blood plasma of a young pig.

TABLE 4. STATISTICS USED IN THE PREDICTION OF THE VITAMIN A CONTENT OF THE PLASMA OF SUCKLING PIGS [a] (mcg. A per 100 ml.)

Week	b_{yx}	Sb_{yx}	$\bar{x}$ Sows	$\bar{y}$ Piglets	s_y	s_x	$s_{y.x}$	$\ddot{Y}$
1	0.609	0.119	40.7	32.4	14.35	16.26	10.56	$0.609X + 7.6$
4	0.473	0.093	33.6	26.7	8.82	12.46	6.33	$0.473X + 10.9$
7	0.341	0.095	34.9	25.1	8.09	13.18	6.83	$0.341X + 13.2$
1-7	0.495	0.066	36.4	28.1	10.78	14.07	8.92	$0.495X + 10.1$

[a] Sb_{yx} is the standard error for the regression coefficient (b_{yx}); s_y is the standard deviation for Y; s_x is the standard deviation for X; and, $s_{y.x}$ is the standard deviation for Y when X is given.

et al. (1941, 1942), Braun (1945), Sobel and Rosenberg (1950) and Merrow *et al.* (1952) that the vitamin A content of the blood plasma is influenced by the dietary intake of vitamin A or carotene. Therefore, it follows that an adequate quantity of vitamin A, or its precursor, in the ration of the sow should ensure a relatively high level of vitamin A in the plasma of her suckling pigs.

Measurement of the vitamin A level in a sow's blood plasma is useful in predicting the level of vitamin A in the blood plasma of her suckling pigs. It would appear that the precision of prediction is greatest when the young pigs are 4 weeks of age, for at this point the standard error of the regression coefficient (Sb_{yx}) and the standard deviation of Y when X is known ($S_{y.x}$) are both at a minimum; the correlation coefficient r_{xy} is approximately the same at that of the first week (0.668:0.690) and considerably larger than that of the seventh week (0.556). The accuracy of the prediction equations may be estimated by comparing $(S_y)^2$, the variation in Y, with $(S_{y.x})^2$, the variation in Y when X is known. Use of the regression equation for the fourth week lowers the original variation by approximately 45% (i.e. $(S_{y.x})^2 \simeq 0.55$ $(S_y)^2$).

Probably the liver stores of vitamin A, with which the young pigs were born, were still influencing the blood plasma levels at one week of age, resulting in considerable variation. When the pigs were 7 weeks old they were consuming appreciable quantities of a creep feed which was fortified with vitamin A. At 4 weeks of age it is probable that the liver stores had ceased to be a major influence and the sow's milk was the principal source of vitamin A, the amount of creep feed being consumed at this age being negligible. At this age, therefore, one would expect a closer relationship between the blood plasma levels of vitamin A in the sow and her young. The age at which young pigs begin to consume relatively large quantities of creep feed would undoubtedly influence the age at which accuracy of predicted vitamin A values decreases.

A regression equation to predict the blood plasma levels of vitamin A in suckling pigs from pooled data obtained at the first, fourth, and seventh week of age, is also indicated in table 4. The value of this equation depends upon whether or not there is a real difference in the relationship at the seventh week compared to the first and fourth weeks. If there is no real difference the pooled regression equation is the most applicable; however, from the data available no definite conclusion can be made in this regard.

None of the suckling pigs showed symptoms of vitamin A deficiency to indicate a critical level of vitamin A in the blood plasma of the pigs or of their dams. The lowest vitamin A level in any sow in this experiment was 8.3 mcg. per 100 ml. of plasma for a very short period. It would seem advisable to maintain a level in excess of this value as Hentges *et al.* (1952) noted that a vitamin A deficiency in young pigs occurred when the level fell below 15 mcg. per 100 ml., although gross symptoms of a deficiency did not occur until the blood plasma level fell below 5 mcg. per 100 ml.

Summary

Data are presented to show that the vitamin A content of the blood plasma of suckling pigs is positively correlated (highly significant) with the level in the blood plasma of their dams. From these data regression equations were formulated which predict the vitamin A level in the blood plasma of suckling pigs from the vitamin A level in the blood plasma of their dams.

The most accurate prediction was made when the suckling pigs were 4 weeks of age, when a regression equation of $\hat{Y}=0.473X+10.8$ was obtained. About 45% of the original variation could be controlled by the use of this equation.

Blood plasma level of vitamin A in the suckling pigs was unrelated to sex.

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GROWTH HORMONE CONTENT OF SWINE PITUITARIES IN RELATION TO GROWTH RATE AND AGE

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GROWTH is the basis of the enterprise of the animal producer and is thus of great importance. The phenomenon of growth is among the most complex in the realms of physiology and nutrition; therefore, a better understanding of the nature of growth means a more intelligent approach to feeding and to genetic selection.

It is well known that growth hormone, directly or through an intermediate, controls the phenomena of somatic growth. Most of this evidence comes from laboratory animals. Because no information is available on the relationship between the rate of pituitary function and the changes which are known to occur in the chemical composition of the soma of pigs as they grow and mature physically, and as nothing is known about the shifts which take place in body composition from so-called market weights to weights in excess of 500 lb., this work was undertaken. From it one should gain information concerning the role of growth hormone in relation to the different phases of typical body growth: the self-accelerating phase, the slower growth rate with approaching physical maturity, and finally, the so-called growth stasis.

It is the purpose of this study to answer some of the questions raised above by sampling a sufficiently large number of representative animals throughout most of the life span and to compare, at stages deemed critical, the growth hormone potency of the pituitary glands with growth rate, the chemical nature of the gain, and finally, with the morphological or skeletal changes which take place.

Experimental

As the interest of this work was primarily centered on the changes which occur in the rate of growth hormone secretion and the changes in the physical and chemical composition occurring during the life span of swine, careful and complete sampling of a representative population

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was essential. This was accomplished by sampling two breeds, Hampshire and Duroc, and by having four general divisions for each breed: fetal, immature, mature and pregnant. These were subdivided so that the fetal group was made up of fetuses aged 56, 70, 84 and 98 days; the immature group was composed of pigs 1, 20, 40, 60, 150 and 225 days of age; the mature group included animals weighing 300, 400, 500 and 600 lb.; and the animals in the 56th, 74th, 84th and 98th days of pregnancy made up the pregnant group. Each age, weight, or pregnancy group was composed of four female animals, two Hampshire and two Durocs.

All post puberal gilts and sows were killed on or about the 10th day of the estrual cycle after they had reached the age or weight requirement of the group to which they were assigned. All other animals were killed when they reached the designated age, weight or stage of pregnancy to which they were assigned. The pituitary glands were collected within 30 minutes after death, properly labeled and immediately frozen. Before they were to be used for assay, they were thawed, freed of connective tissue, and weighed. The anterior lobe was separated, weighed, macerated between glass plates, dried under vacuum in a calcium chloride desiccator, ground to a fine powder, and stored in a desiccator until used.

The carcasses were split down the back, and the right side of the carcass was retained for chemical analysis along with the internal organs and the head. Samples were taken from the carcass, the head and offal so that the total body composition could be determined. At the time of sampling, all components of the body were carefully weighed, and the length of the femur and humerus were obtained. All samples were analyzed for dry matter, ether extract and nitrogen, but ash determinations were made only on bone samples.

Assay Methods

While Baird (1951) furnished a good estimate of the amount of swine anterior pituitary tissue necessary to evoke a satisfatcory measurable increase in the width of the epiphyseal cartilage of hypophysectomized female rats, several preliminary trials were conducted to establish a satisfactory daily dose. One hundred micrograms per day were found to be optimal for the present experiment, because it permitted variations in hormone potency of individual glands from animals of various ages to be measured satisfactorily. The criteria used for assaying the growth hormone content of pituitaries were the increase in body weight and the width of the epiphyseal cartilage of the assay animals, which

were hypophysectomized at approximately 21 days of age and were received at the laboratory one day post-operative. The animals were weighed daily and any rat gaining more than 7 gm. in 8 days was suspected of being incompletely hyphysectomized and discarded.

As all of the glands could not be assayed simultaneously, they were assayed in series to which the glands were allotted at random so that the entire range of ages and stages of pregnancy was included in each assay series. The assay procedure for the individual glands was carried out in a manner similar to that employed in the preliminary trials with the exception that a group of positive control rats was used. These rats received daily doses of 100 mcg. of an homogenous crude pituitary powder which was available in sufficient quantities to be employed as a standard throughout the trials. This was done in order to bring out any differences in response of the assay animals from series to series.

Results

Pituitary Growth Hormone Assays

The results obtained from the pituitary assays are summarized in table 1. They can be expressed in three different but related ways, namely: unit potency, total potency, and the amount of available hormone, depending on the aspect of the study on which interest centers. Unit growth hormone potency of the A.P. is the response elicited (measured by the increase in width of the epiphyseal cartilage) by a given quantity of pituitary tissue. On the basis of unit potency, all pituitaries used in this study were directly comparable because the daily dose of 100 mcg. of pituitary tissue was used in all cases. Pituitary gland weight varied between animals of different weights and between animals of the same weight. Hence the total amount of hormone contained or secreted by the whole pituitary gland was calculated by multiplying the unit potency by the dry weight of the anterior lobe. While total potency of the gland is valuable for some comparisons, it may be misleading in others. For example, two animals having the same total pituitary potency cannot be presumed to be stimulated by the growth hormone to the same extent if one animal is larger than the other. To make comparisons regardless of size of animal possible total potency was divided by the body weight of the donor animal. The resulting figure is called available hormone, and it is thought to represent the quantity of pituitary hormone which is available to a unit of the animal's body mass. The major error in this calculation results from different degrees of fatness of different animals if blood volume is considered a

constant percentage of body weight. For this reason, it was thought useful to compute the quantity of available hormone per unit of body protein in some cases.

Statistical analysis of the data showed that the unit potency of the pituitary glands was significantly ($P<.01$) lower in animals less than 20 days of age than it was in older donor animals (table 1). The total

TABLE 1. ABRIDGED SUMMARY OF GROWTH HORMONE ASSAYS (FOUR ANIMALS PER GROUP)

Group	Dry anterior pituitary weight	Unit potency	Total potency	Total potency per kilo body weight	Total potency per kilo body nitrogen	(A.P. per kilo body weight) $\times 10^8$
	mg.					
A. Fetal						
56 days[a]	1.4[e]	15.5**	21.7	34.7	.....	.....
70 days[b]	2.2[e]	19.1**	32.3	41.3	.....	.....
84 days[b]	4.5[e]	13.7**	65.5	38.0	.....	.....
98 days[c]	3.3[e]	23.3**	91.7	50.7	.....	.....
B. Immature						
1 day[d]	2.5	20.3**	44.3	49.1	2978.6	276.2
20 days	5.1	38.6	205.0	52.5	2621.3	134.4
40 days	9.8	46.7	432.5	44.8	1903.9	94.6
60 days	17.9	26.3	454.5	23.4	1175.4	93.0
150 days	35.7	39.7	1411.7	20.2	1067.7	51.3
225 days	39.0	21.2	826.1	8.0	440.9	37.4
C. Mature						
300 pounds	37.8	29.4	1098.5	8.3	488.0	28.5
400 pounds	80.9	32.1	2523.6	13.8	752.4	43.6
500 pounds	74.3	21.2	1641.8	7.2	437.6	32.4
600 pounds	108.8	26.9	2892.9	10.6	665.6	40.0
D. Pregnant[f]						
56 days	89.3	23.8	1818.6	8.8	.....	39.8
70 days	51.3	27.8	1276.1	9.0	.....	32.1
84 days[d]	41.9	35.0	1509.0	10.3	.....	29.3
98 days	52.5	36.0	1876.3	12.6	.....	35.2

[a] Female fetuses from one litter.
[b] Female fetuses from three litters.
[c] Female fetuses from four litters.
[d] Only three animals.
[e] Total average A. P. weight of all female fetuses.
[f] Not comparable to A., B., and C. because of age differences.
** Significantly different from rest ($P<.01$).

potency was seen to be low in fetal pituitaries, increasing rapidly throughout the life of the animal. However, the available circulating hormone was high in pigs younger than 20 days of age, decreased abruptly to the age of 300 days and then plateaued, remaining unchanged throughout the older ages. The significance of these changes will be discussed together with the physical and chemical changes occurring in the carcasses of the donor animals.

Neither breed nor stage of pregnancy had any effects on pituitary potency.

Chemical and Physical Measurements of Donor Animals

The femur and humerus increased in length rapidly and at a comparable rate until the animal reached about 150 days of age when the rate of increase in length of the humerus became less rapid that that of the femur. At the age of approximately 320 days, the rate of increase

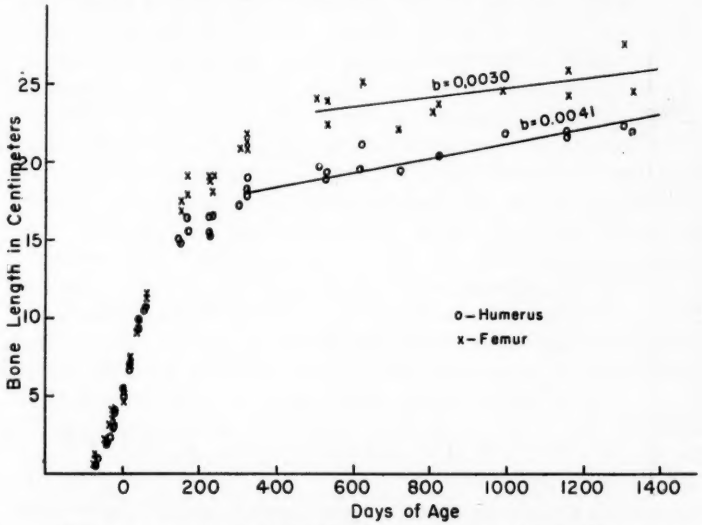

Figure 1. Comparison of growth rates of two long bones with age.

of the humerus had lessened to a considerable extent, but this bone continued to increase linearly at a significant ($P<.01$) rate of 0.0041 cm. per day through the remainder of the age range covered (Figure 1).

Determinations of total dry matter, ether extract, nitrogen, and bone ash of the immature and mature animals showed an increase in the actual amounts of these constitutents throughout the age span covered in this study. Dry matter and ether extract increased at a comparable rate, dry matter comprising about 18% of the body weight in the day-old animals increasing to approximately 58% in animals 1300 days old. Ether extract made up a negligible portion of very young animals and increased until it comprised 50% of the total body weight.

Crude protein increased after birth from about 10% to 13% of body

weight at 45 days of age. Thereafter the percentage of crude protein declined slowly to a constant level of 10% at approximately 300 days of age. Bone ash decreased from 4 to 2% of body weight in the first 60 days of life and became constant, comprising approximately 2% of the body weight of the animals from 60 to 1400 days of age.

The weight of the anterior pituitary increased rapidly until the age of about 225 days, after which the increase continued at a lower rate. The ratio of anterior pituitary weight to body weight decreased rapidly until the age of 225 to 300 days when it became constant (Figure 2).

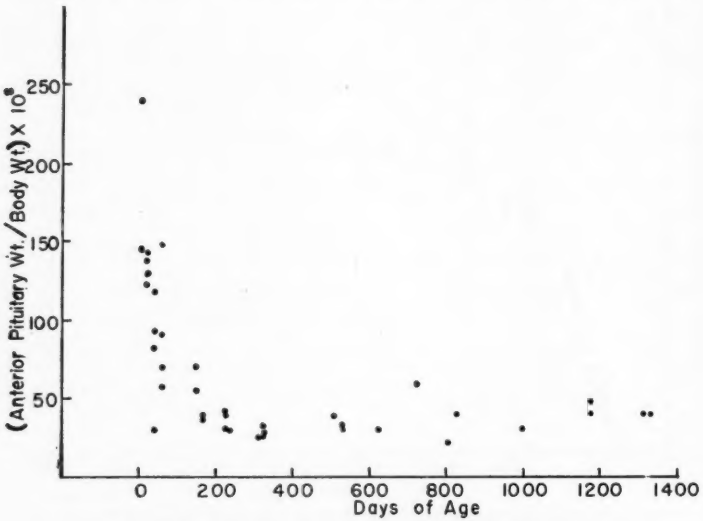

Figure 2. Comparison of pituitary weight with age.

Discussion

The data obtained in this study can logically be discussed under three different headings: the relation between growth hormone content of pituitary glands and the total increase in body weight of the donors, the relation to bone growth of the donors, and that to changes which occur in the amount of protein, fat and water deposited in the body of the donors at the various ages with which this study is concerned.

The pituitaries of fetuses and of pigs less than 20 days of age contained significantly lower amounts of growth hormone per unit weight of gland than did older animals. After 20 days of age, the unit po-

tency became constant and remained so regardless of whether the weight changes were slow or rapid. This suggests that in pigs older than 20 days, a constant amount of pituitary tissue produces the same quantity of growth hormone, irrespective of age or the rate at which the animals are growing. It is difficult to understand why there is acceleration of growth rate in young animals in the absence of an increased rate of growth hormone secretion. This difficulty can be partly resolved if it is remembered that the ratio of pituitary gland weight to body weight is higher in younger than in older animals (Figure 2). If the total amount of hormone contained in the pituitary gland is determined, it is found that this figure increases rapidly beginning with 20 days of age and remains high throughout the life span of the pig, including the rapid and slow phases of growth. While this explains growth acceleration it does not account for deceleration at maturity, at which time the total potency is still high. The amount of growth hormone per unit of body weight between 20 and 300 days of age (phase of most rapid growth) was substantially greater than that during the remainder of the growth period (Figure 3). This suggests that deceleration of growth results as the amount of available growth hormone drops below the point which is essential for rapid and vigorous growth. This may also account for the fact that growth deceleration occurs even though no actual stasis was ever reached in the group of animals with which we dealt, even in those reaching the age of 1300 days.

These results confirm the theory of Baird *et al.* (1952) who conducted a similar study on pigs between 56 and 225 days of age and concluded that growth stasis followed "dilution" of the available hormone to the point where the body can no longer respond by vigorous growth to the lowered concentration.

A similar situation was found for the thyroid stimulating hormones (TSH) determined by Hollandbeck (1955) on the same pituitaries on which the growth hormone potency was established. Here too the self-accelerating phase of the growth curve coincided with the time in which the larger amounts of thyrotrophic hormones were available, and the flattened part of the growth curve coincides with time in which a decreased amount of thyrotrophic hormone is available per unit of body weight. Because growth hormone and TSH are known to have a synergistic effect on growth (Smith, 1933; Evans *et al.*, 1939), and the titer of these hormones follow similar trends, it seems probable that the changes in the total available growth and thyrotrophic hormones fully account for phenomena of the normal growth curve of swine.

We now turn to the two factors which are considered true criteria of growth, namely increase in bone length and increase in nitrogen deposition. The data show that increase in the length of the long bones depends largely on the amount of available growth hormone per unit of body weight, and that there is a similar relation between the rate of nitrogen deposition and the decrease in titer of total available hormone per unit of body nitrogen (Figure 4). Although the rates of bone growth and of nitrogen deposition were greatly reduced after the first

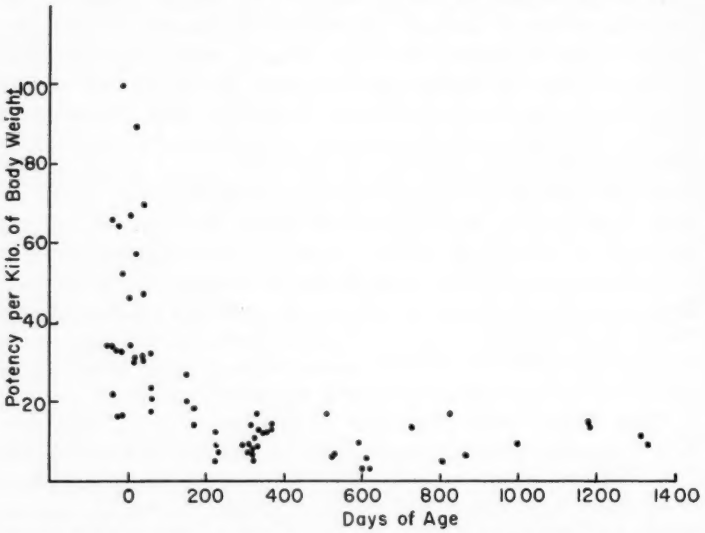

Figure 3. Comparison of available growth hormone (total potency of gland divided by body weight) with age.

300 days, there was a continued slow increase in these components. Therefore, we conclude that, though rapid growth ceased at about 300 days of age, there was a continued steady but slower increase in both bone length and nitrogen deposit (e.g. "true" growth) throughout the remaining period covered in this study. The rate of growth of the humerus and the femur should be noted. The femur continued to increase in length rapidly even past the age at which the length of the humerus first began to plateau and the titer of the available growth hormone had become constant in the relation to body weight. This difference could be explained on the assumption that the sensitivity of these two

bones to hormone stimulation differs (as has been shown by Simpson *et al.*, 1950), and hence that different bones respond to growth hormone stimulation by different rates of growth.

Finally, considering the changes which occur in the chemical composition of the nonskeletal portion of the carcass, in general the pigs weighing less than 400 lb. behaved in accordance with expectation and in agreement with previous data obtained by Mitchell and Hamilton (1929) and Hogan *et al.* (1925). However, it is interesting that in

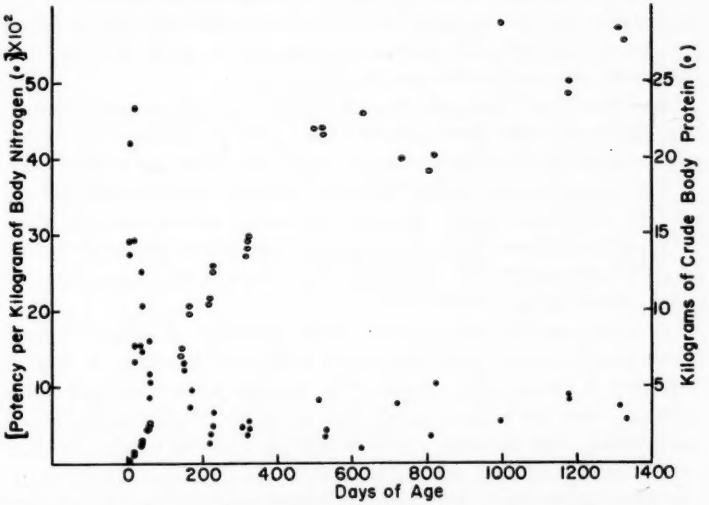

Figure 4. Available growth hormone in relation to body nitrogen, body protein and age.

pigs which weighed over 400 lb., there was continued protein synthesis in addition to the deposition of fat and water. The most rapid rate of fat and dry matter formation coincided with the most rapid rate of true growth and proceeds well beyond the time when the rate of protein deposition had plateaued.

Summary

Anterior pituitary glands from 65 female Hampshire and Duroc swine were assayed for growth hormone content using two criteria of their effect on immature hypophysectomized female rats: (1) increase in width of the proximal epiphysis of the tibia, and (2) gain in weight.

Pituitary glands were collected from animals belonging to the following categories: fetal, immature, mature and pregnant. Each category was subdivided into four to six sub-groups and included a range of weights from fetal to 600 lb. Chemical analyses and long-bone measurements were made on all experimental animals included in the immature and mature phases.

Dry matter, ether extract, nitrogen, and bone ash of the bodies of non-pregnant animals showed an increase in the total amounts of these constituents throughout the age span (1–1400 days of age) covered in this study. Dry matter, ether extract, protein, and bone ash increased at a rapid rate until the animals reach an age of about 300 days and thereafter continued to increase at a slower rate.

The femur and humerus showed a rapid increase in length during the phase of rapid body growth. After growth slowed, these bones continued to increase significantly in length up to the age of 1400 days.

The weight of the anterior pituitary increased rapidly until the age of 225 days, after which the rate of increase slowed but continued. The ratio of anterior pituitary weight to body weight decreased rapidly until the age of 225–300 days when the ratio became constant for the remainder of the ages studied.

Growth hormone potency of a given quantity of pituitary tissue (unit potency) was significantly lower in animals less than 20 days of age than it was in older animals. The total hormone contained in the pituitary was low in fetal glands and increased throughout the life of the animal. The hormone available per kg. of body weight was high in young animals, decreased to the age of 300 days, and thereafter remained constant. No differences in pituitary potency could be ascribed to breed or reproductive state.

It is concluded that reduction in growth rate at 225 days of age is due to dilution of available circulating growth hormone to the point where the body no longer responds by vigorous growth to the low concentration.

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GONADOTROPHIC HORMONE CONTENT OF SWINE PITUITARY GLANDS IN RELATION TO AGE

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THE work reported here forms a part of a more extensive project which was designed to study changes in concentration of various hormones of pituitary glands of pigs from fetal stages, through the active growth phase, puberty, sexual activity and the period of almost complete growth stasis. The present paper is concerned with the concentration of gonadotrophic and thyrotrophic hormones during these phases. It was of interest to find out what changes occurred in the concentration of gonadotrophic hormone during prepuberal stages and especially at the time when sexual maturity is reached.

Materials and Methods

Study of the available literature dealing with gonadotrophic hormone potency of pituitary glands emphasizes the lack of agreement between the different reports. At least part of this diversity of opinion is due to the fact that frequently materials for assay came from a few odd specimens without regard to age, weight or reproductive stage. In the present study the animals furnishing the glands for assay had a known history and were killed according to a carefully considered plan at intervals at which the information furnished was thought to be of maximal value.

Fifty-seven sows and gilts of various ages were obtained from the University of Illinois swine herd, weighed individually and transferred to lots suitable for their size and number. They were self-fed to supplement alfalfa pasture, sheds and automatic waterers being provided at all times.

Where numbers permitted, sows and gilts were randomly assigned to the experimental groups. Where pertinent, heat was checked twice daily with a vasectomized boar in order to establish estrous cycles. All sows

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[2] We are grateful to S. W. Terrill for making this study possible.

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and gilts with cycles of abnormal lengths were excluded from the experiment.

Two breeds, Hampshires and Durocs, were used in these experiments and each breed consisted of three general divisions: immature females growing rapidly, physically mature females and pregnant females. These divisions were further subdivided so that the immature group was composed of pigs 1, 20, 40, 60, 150 and 225 days of age; the mature group included animals weighing about 300, 400, 500 and 600 pounds; and animals in the 56th, 74th, 84th, and 98th days of pregnancy made up the pregnant group. Each age, weight or pregnancy group was composed of four female animals, two of each breed. The postpuberal, non-pregnant gilts and sows were slaughtered on about the tenth day of the second cycle after they had reached the age or weight requirements of the experimental group to which they had been assigned.

The gilts and sows belonging to the "pregnant group" were bred to fertile boars at the second heat or rebred at the next heat period. These animals were slaughtered within four to five days of the time assigned to the experimental group to which they belonged.

In all cases pituitary glands were removed from the head as soon after the latter became available after the animal had been killed, usually within 30 minutes after death. The glands were placed in individual vials, properly identified, frozen and kept frozen until they were ready for use. The reproductive organs and the thyroid glands of all animals regardless of age were described, measured and weighed.

Day-old male chicks were chosen for the assay of thyrotrophic and gonadotrophic hormones because both can be assayed simultaneously on the same chick. The response of the two end organs, testes and thyroids, to hormone action on them is independent of each other. The chicks used were received at the laboratory the day following hatching from the University of Illinois Poultry Laboratory. They were kept in standard chick brooders and were fed an acceptable growing ration.

Before pituitary glands were to be used for assay they were allowed to thaw and stalk and adhering connective tissue were removed. Each pituitary was weighed on a Roller-Smith Torsion Balance, the pars nervosa was removed and the anterior lobe was reweighed, placed between two glass plates and crushed by an appropriate amount of pressure. The glass plates with the adhering mangled tissue were separated, immediately placed in a calcium chloride desiccator and dehydrated under vacuum at room temperature. Dehydration was completed in from 36 to 48 hours.

The dry glands were scraped from the plates and reweighed so that the wet weights of the whole gland and the anterior lobe, and the dry weight of the anterior lobe were available for each gland. The dry glands were ground into a very fine powder with a mortar and pestle and stored in a calcium chloride desiccator until ready for use. Before injection, the exact amount of powder to be used was weighed, placed in a vial and suspended in enough distilled water to permit injection. This aqueous suspension was kept in the refrigerator during the 4-day period of injection.

Where amount of pituitary powder permitted, each gland was assayed individually. Pituitaries of younger gilts yielded insufficient amounts of powder for individual assays and powder from gilts within the same experimental group was pooled for assay. To avoid genetic and seasonal variations in sensitivity of the assay animals, all glands were assayed simultaneously on chickens of a single hatch. In this experimental design the variability found can be ascribed to changes in hormone content of glands during growth or during gestation and variation due to test animals is of negligible importance.

Preliminary assays showed that a total dose of 10 mgs. of powder from physicaly and sexually mature hogs produced optimal responses in the end-organs to be used in the assay. The weight-increases of testes and thyroids of assay animals lay in the middle range of the assay curve thus permitting fluctuation in end-organ weights in response to individual potencies of glands to be assayed. Because all glands were to be assayed simultaneously and because some glands yielded less than 30 mg. of powder, it was decided to let the amount of powder available determine the number of chicks, up to a maximum of three, to be used for each gland. Thus, in some cases, there was enough material for only one chick, while with heavier glands, a maximum of three chicks was used.

The birds were two days old at the time of the first injection. A total of 10 mg. of pituitary powder per chick was injected subcutaneously in eight equal doses during four days. At each injection a chick received the powder suspended in 0.50 ml. of distilled water. The birds were killed on the fifth day, about 12 hours after the last injection. Testes were removed, cleaned, and weighed on a Roller-Smith Torsion Balance. Thyroids were removed, fixed in Bouin's solution, cleaned and then weighed. Comparison of weights of testes and thyroids of chicks injected with pituitary tissue with weights of these organs from chicks injected with distilled water served as the measure of hormonal effects.

Results and Discussion

Results obtained can be logically expressed and discussed in three different ways. Weights of testes and thyroids permit comparison between hormone potency of a given amount of pituitary gland tissue. This is called the "unit potency" of a given pituitary gland. Other things being equal, this measure permits direct comparison of changes in hormone content of a given quantity of pituitary tissue between animals, and in relation to age or reproductive state of donors. Because the pituitary gland as well as the whole animal increase in weight throughout the span of this experiment, and because these rates of increase may differ, it is logical to find the "total hormone potency" of the gland.

TABLE 1. ABRIDGED SUMMARY OF GONADOTROPHIC HORMONE ASSAYS (NON-PREGNANT FEMALES)

Age	Body weight	Ovary weight	Dry weight of anterior pituitary	Unit potency	Total potency	Available hormone	Hormone available to ovary
days	lb.	gm.	mg.				
33	25	0.18	12.5	45.8	464.39	21.25	211.58
153	123	5.73	32.6	40.4	1346.24	10.86	2.03
228	230	11.40	38.9	33.9	1332.70	5.79	0.54
317	293	14.58	37.8	37.9	1441.62	4.94	0.34
523	435	12.08	69.5	31.6	2526.27	5.05	0.50
620	531	25.60	73.8	29.5	2177.10	4.10	0.16
730	417	19.30	67.0	19.5	1206.50	3.13	0.16
810	436	23.10	63.8	32.0	2074.48	4.90	0.27
1000	605	34.49	82.8	20.4	1689.12	2.79	0.08
1180	550	19.40	113.4	25.6	2768.78	5.09	0.26
1320	613	21.20	111.8	19.2	2151.10	3.51	0.16

This is defined as the product of "unit potency" and the weight of anterior pituitary gland. Finally, it was of interest to compare hormone potencies of different glands regardless of size of animals. This was made possible by determining the amount of "available hormone" which, was obtained by dividing "total hormone potency" by total weight of the end organ on which it acts, e.g. body weight, ovary weight or thyroid weight.

Pituitary Gland Gonadotrophic Potency of Growing Animals

Statistical analysis showed that there was no significant difference between gonadotrophic potencies of pituitary glands from non-pregnant Hampshire and Duroc females. For this reason the assay results were pooled and they will be discussed without regard to breed (table 1). Our data agree with results obtained by other workers in that a highly significant positive correlation was found between body weight and

age (b=.39; r=.91), although this relationship is obviously curvilinear (figure 1). Similar calculations showed that the dry weight of pituitary glands increases linearly with age (b=.07; r=.89; figure 1).

The unit potency of gonadotrophic hormone decreases very significantly (P<.01) and linearly with increasing age (figure 2). Using two-place logarithms of assay results themselves to minimize the variation in response elicited by crude anterior pituitary powder, it was

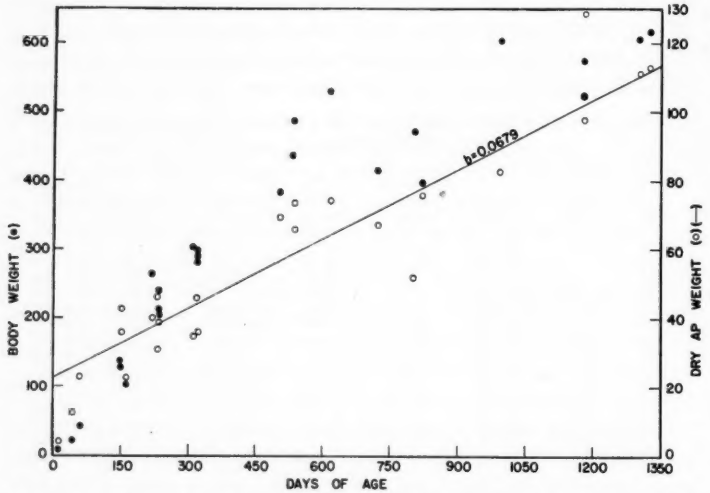

Figure 1. Comparison between body weight and pituitary weight (dry anterior lobe) with age.

found that the correlation with age is —.67 and with body weight, —.68. From these data it is seen that the gonadotrophic hormone output per unit of pituitary weight decreases with age and mildly suggests that the pituitary gland may be increasing in size at a more rapid rate, or that the composition of the gonadotrophic complex (proportion of FSH and LH) may be changing which could also account for decreasing unit potency. The type of assay employed does not differentiate between the proportion of the two hormones making up the complex. When total hormone potency was calculated, it was also found to be increasing significantly (P<.01) and linearly with increasing age (figure 2) and to be correlated with age (r=.63) and with body weight (r=.66). These relationships show that the increase in size of pituitary glands

occurs at a faster rate than does the decrease in rate of hormone output per unit of pituitary tissue. This, of course, does not rule out the possibility of a qualitative change in the gonadotrophic complex.

It has been commonly assumed that with increasing age of females during the prepuberal period, there is a gradual increase in the gonadotrophic hormone potency of their pituitary glands. It is known that

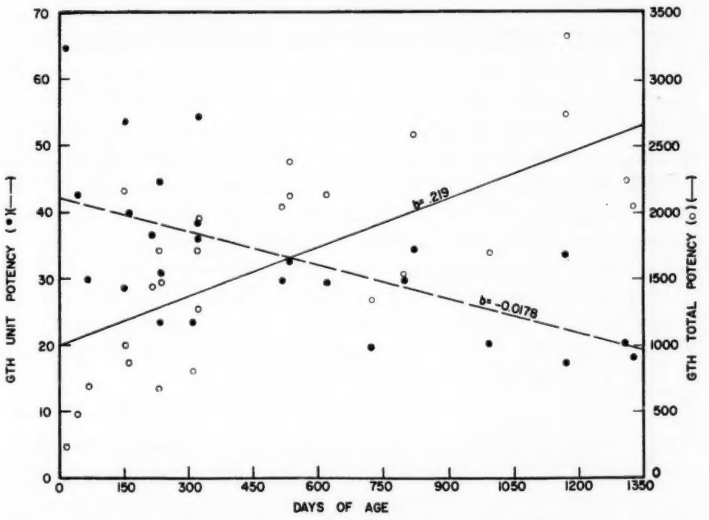

Figure 2. Comparison between unit potency and total potency of gonadotrophic hormone with age.

some months prior to puberty, follicular growth is initiated and "waves" of follicles grow and recede. The increasing number and size attained by follicles at each successive "wave" was thought to be due to a corresponding increase in the titer of gonadotrophic hormones in the blood stream. It was further assumed that at puberty the titer of gonadotrophins becomes sufficiently high to cause follicles to grow to ovulatory size, to ovulate and to establish the regular cyclic pattern of the pituitary-ovarian relationship. While this theory seems plausible, no attempt has been made to check its accuracy by correlating the concentration of gonadotrophic hormone with age, using a homogenous group of animals of sufficiently diverse ages.

Contrary to expectation this experiment showed that the amount of

hormone available per unit of body weight is very high in day-old females and that there is a rapid decrease in hormone potency to about 220 days of age after which it remains constant through 1330 days of age (table 1 and figure 3). It is considered significant that the onset of puberty coincides with the lowest titer of available hormone. The picture is even more dramatic when the amount of available hormone per unit of ovary weight is correlated with age (table 1).

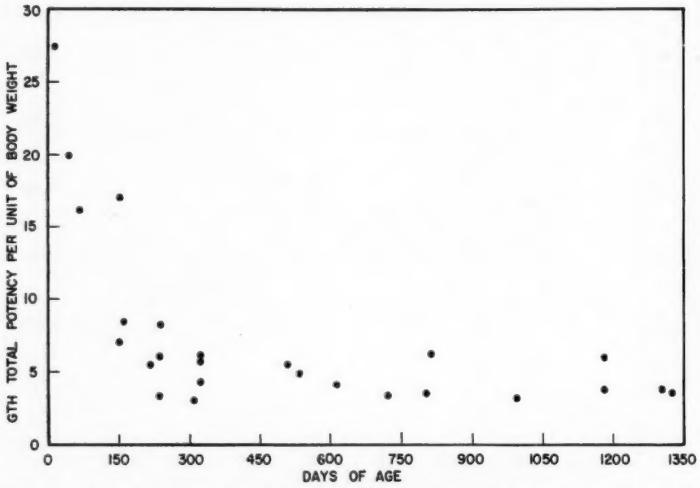

Figure 3. Comparison between available gonadotrophic hormone (total potency divided by body weight) with age.

This apparent drop in available hormone from birth to puberty may be due to an actual decrease in the rate of hormone secretion, but it is more likely to be due to a shift in the proportion of the two hormones comprising the gonadotrophic complex. Unfortunately no techniques are available at present which would permit assay of these two hormones using such small quantities of hypophyseal tissue as are available from individual pituitary glands.

On the basis of these results the theory is advanced that the onset of puberty is not due to a gradual increase in the rate of secretion of the gonadotrophic complex but is actually dependent rearrangement of the proportion of FSH and LH and a decerase in the output of the complex by the pituitary gland. While no data are available on the changes which occur in the proportions of FSH and LH during this crucial

period, the theory seems plausible that at the time of birth the hypophysis secretes predominantly FSH thus accounting for the high gonadotrophic potency of that gland. With increasing age of the female there may be an actual decrease of the rate of secretion of the whole complex, or a decrease of FSH secretion coupled with an increase in the rate of LH secretion. The latter relationship would account for increasing ovarian activity which finally culminates in cyclic ovarian function with successive heats and ovulations.

The morphological examination of ovaries of animals from which pituitaries were obtained for assay shows that by 150 days of age ovarian follicles have attained ovulatory size but that not a single ovulation had occurred at that time. At that age the amount of available gonadotrophic hormone is still significantly higher than it is at 225 days of age when the first ovulations occurred. It is postulated that at about this time (225 days) FSH and LH reach the proper ratios for the first time, permitting onset of puberty and subsequent cycling behavior including heat and ovulation. The age at puberty observed in this study is in line with that reported by Phillips and Zeller (1943), Rice and Andrews (1951), Robertson *et al.* (1951), Burger (1952) and unpublished data from this station.

Pituitary Gland Potency of Pregnant Animals

During pregnancy the unit potency as well as the total gonadotrophic potency of pituitary glands from the two breeds differ significantly ($p<.05$), the Hampshires having more potent pituitary glands.

The results of this experiment show that the gonadotrophic potency of pituitaries of sows is high in early pregnancy followed by a very gradual decline until shortly before parturition. While this decrease in potency with advancing stages of pregnancy could not be shown to be statistically significant, the downward trend was similar in both breeds (Hampshires, $b=-.27$; Durocs, $b=-.12$). Small numbers probably account for lack of statistical significance. Declining gonadotrophic potency of pituitaries of pregnant females was also noted by Robinson (1950) in the sow, and Nalbandov and Casida (1940) in the cow. This decline in potency may be explained by the well known ability of estrogen (of placental origin in this case) to have restraining effect on the formation of gonadotrophic hormones by the pituitary gland.

Correlations between litter size and total pituitary potency of gonadotrophic hormone showed a definite breed difference (Hampshires, $b=0$; Durocs, $b=1.94$, $P<.05$). This difference may be due to the fact

that the pregnant Hampshires were predominantly gilts, whereas, the Durocs were predominantly mature sows. It is well established that with increasing age there is a corresponding increase in litter size up to, and including the fourth litter. This factor, along with increased total potency of gonadotrophic hormone of pituitary glands with increasing age, may erase any major "true" breed differences.

The sex ratio of the litters failed to show any significant effect on the total gonadotrophic hormone potency of the dams' pituitaries.

There was a highly significant positive correlation between the total pituitary potencies of the gonadotrophic and thyrotrophic hormones (Hollandbeck, 1955).

Summary

Pituitary glands of 26 Hampshire and 20 Duroc females varying in age from 1 to 1330 days and including cycling and pregnant animals, were assayed for gonadotrophic hormone content. The results were correlated with age and body weight.

No significant difference in hormone potency was found between breed means of open sows. The unity potency of gonadotrophic hormone decreased linearly with increasing age ($P<.01$). Total potency for open sows showed no difference in breed means and increased linearly with age ($P<.01$).

The amount of available hormone per unit of body weight was very high in day old females, decreased rapidly to about 225 days when first heats and ovulations occurred, and thereafter remained constant through 1330 days of age.

Contrary to current assumption, onset of puberty coincides with the lowest titer of available gonadotrophic hormone. The theory is advanced that the high potency of glands from young animals is caused by a high rate of FSH secretion. Gradually the FSH/LH ratio of the complex becomes adjusted and when the proper ratio is reached cyclic ovarian function begins at puberty between 225 and 300 days. The low gonadotrophic potency of the pituitary gland is retained throughout the remainder of the reproductive life of the females (up to 1300 days of age included in this study).

In pregnant sows there is a significant ($P=.05$) difference in gonadotrophic hormone potency between breeds, the Hampshires giving an 11.6 mg. greater chick testes weight. While the decrease in total gonadotrophic hormone potency with increasing length of pregnancy could not

be shown to be statistically significant, the downward trend was similar in both breeds (Hampshires, b=.22; and Durocs, b=.12).

Correlations between litter size and total pituitary potency of gonadotrophic hormone showed a definite breed difference (Hampshires, b=0; Duroc, b=1.94; $P<.05$). This difference may be due to differences in the age of females compared, rather than to a real breed difference.

The correlation between pituitary gland potencies for gonadotrophic and thyrotrophic hormones was positive and highly significant ($p<.01$).

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USES OF ASPIRATION BIOPSY TECHNIQUE

E. S. Erwin, I. A. Dyer, T. O. Meyer and K. W. Scott

State Department of Washington, Department of Animal Husbandry, Pullman

OBTAINING tissue samples from live animals provides an excellent method for histological, biochemical, and physiological studies without sacrificing the experimental animal. The aspiration technique has been used by many workers to obtain liver samples. This technique of liver biopsy in farm animals was first developed by Dick (1944) in Australia for copper studies in sheep. His method was adapted from the technique devised by Iverson and Roholm (1939) for human medicine. The technique of aspiration biopsy of the liver was adapted to cattle by Garner (1950) who reported a maximum success of 90% in obtaining samples. Others have since modified instruments and techniques. The method developed by Whitehair *et al.* (1952) involves an incision of 1.5 inches, in the twelfth intercostal space about 6 to 8 inches from the dorsal median plane. The liver was located with the aid of a modified flashlight before the 1.5 to 2.5 gm. sample was removed. This method proved time-consuming as well as deleterious to the rates of gain measured in the test animal. Negative pressure obtained from a syringe attached to a cannula was used by Loosmore and Alcraft (1951) to obtain liver samples. However, a 0.4 gm. sample was the average weight obtained and 15 minutes were required per sample. This technique was refined by Seghitti and Marsh (1953) whose technique and instruments were further refined by Bone (1954). Most workers have presented techniques in which samples were withdrawn from docile animals.

This report includes modifications of instruments developed by others and procedures of three techniques (liver biopsy in beef cattle and young pigs, and back-fat sampling of swine) which can be employed with the same instrument.

Instruments used in the biopsy techniques described herein are shown in figure 1. The essential difference between the instruments shown and those described by Bone (1954) and Seghitti and Marsh (1953) is in the cannula attachment, the absence of dosage nut on the Record syringe, and the absence of a depth stop on the cannula.

The cannula was constructed from a stainless steel tube 16 cm. long

with an inside diameter of 8 mm. The trocar was constructed from a solid stainless steel rod, which fits with a minimum clearance inside the cannula so that the four levels at the point match with the serrated edges of the cannula. Sharpening of the instruments is facilitated by placing the trocar inside the cannula.

A 20 ml. modified Record syringe is shown in figure 1. The original needle attachment was removed and replaced with a brass tooled lock-type cannula attachment. This attachment was constructed with a rubber gasket and a lock mechanism so that when the cannula is at-

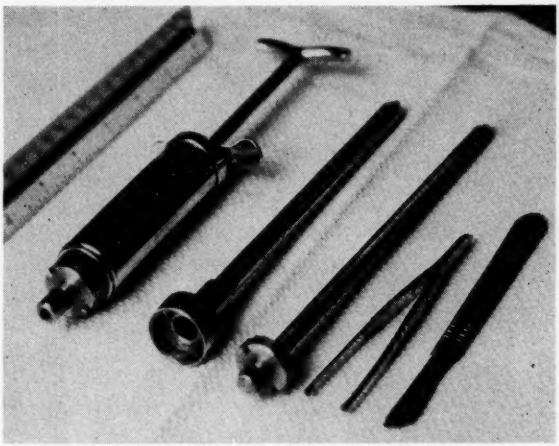

Figure 1. Instruments used in biopsy techniques.

tached by a quarter turn to the syringe, no pressure can be lost from the syringe. Other workers who have reported instruments used for liver biopsy techniques (Dick, 1944; Seghitti and Marsh, 1953; and Bone, 1954) reported the use of slip-type syringe-cannula adaptors. We at this station first constructed our biopsy instruments in this manner. Unsatisfactory results were obtained because occasionally the negative pressure was lost, and consequently the sample could not be extracted, especially when the animal became excited during the operation.

The finger grips and the dosage nut were removed from the original syringe. A small slot was cut in the head of the syringe to permit the passage of a rod attached perpendicularly to the plunger handle. Maximum pressure could be obtained by pulling the plunger back and setting the small rod. This increased the efficiency and speed of operation.

Liver Biopsy in Beef Cattle

The area of operation is shaved free from hair and disinfected. An incision, approximately one-half inch, parallel to the ribs is made by a quick thrust with a scalpel in the twelfth intercostal space about six inches from the dorsal median plane on the right side of the animal

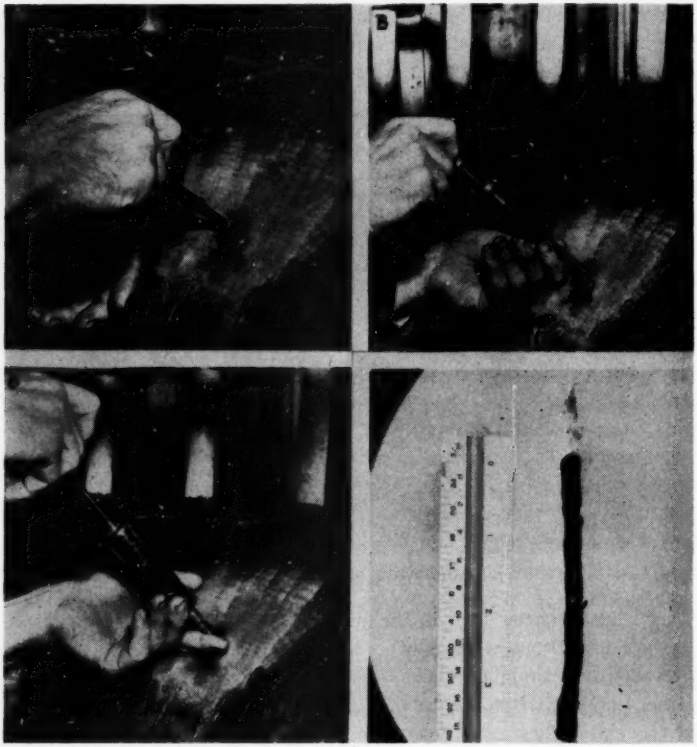

Figure 2. Procedures for liver biopsy in cattle. A. point of incision. B. trocar being removed from cannula. C. syringe attached to cannula with negative pressure applied. D. typical liver sample obtained.

(figure 2A). A simple and rapid method for determining the point of incision is for the operator, with his left hand, to locate the last rib. Then, proceeding anteriorly, he finds the twelfth intercostal space and spans the distance with his thumb and index finger to the backbone; this is the point of incision.

The scalpel is replaced in 70% ethyl alcohol and the sterile trocar and cannula are removed and rapidly inserted through the incision. After the trocar and cannula are through the skin and musculature, the liver is located by pointing the instrument ventrally at an angle of 45 degrees with the horizontal plane and anteriorly 45 degrees with the saggital plane. The location of the liver seems to vary between animals; however, as the operator becomes skilled, he can locate the liver with the trocar cannula by the texture.

The trocar is then removed rapidly (figure 2B), being careful not to lose position with the cannula. The syringe is rapidly attached to the cannula and inserted into the liver about two to three inches further with a rotating motion (figure 2C). The plunger is withdrawn, causing negative pressure, and the syringe and cannula are removed rapidly.

The sample is then removed from the cannula by releasing pressure of the syringe into a beaker of physiological saline solution. After the sample is washed free from blood, it is dried on filter paper and frozen. Figure 2D shows a representative sample that can be obtained by this technique. Samples have averaged about 1.0 to 1.25 gm.

Discussion

Most workers who have reported techniques of liver biopsy have used docile animals, usually dairy animals. Fifteen minutes or more were reported as the minimum time required to secure a sample after the animal has been restrained. Since the experiments at our station required liver samples from large numbers of range animals, it was necessary to develop a more rapid method. It has been found that an experienced operator can obtain a liver sample within one minute after the animal is restrained.

No deleterious effect from the liver biopsy technique has been noted. These studies have also involved the records of feed consumption, in which case no animals have been observed to "go off feed" as a result of biopsying. Moreover, as many as four liver samples have been obtained from one animal at one time, and as many as five operations have been conducted at different times on given animals as close as five days apart with no apparent ill effects.

When liver biopsy was first attempted at this station, procaine was used to anesthetize the operation area. This procedure was not only time-consuming but also resulted in exciting the animal more than the actual biopsy. We therefore discarded the use of any anesthetic. It must be emphasized that all procedures should be conducted as rapidly as possible.

Liver Biopsy in Young Pigs

The pig was first anesthetized with an intrathoracic injection of sodium pentobarbital. Ether was then used to keep the animal under desirable anesthesia. The hair was shaved and the area of incision was washed and disinfected. An incision approximately 2.5 cm. parallel to the ribs in

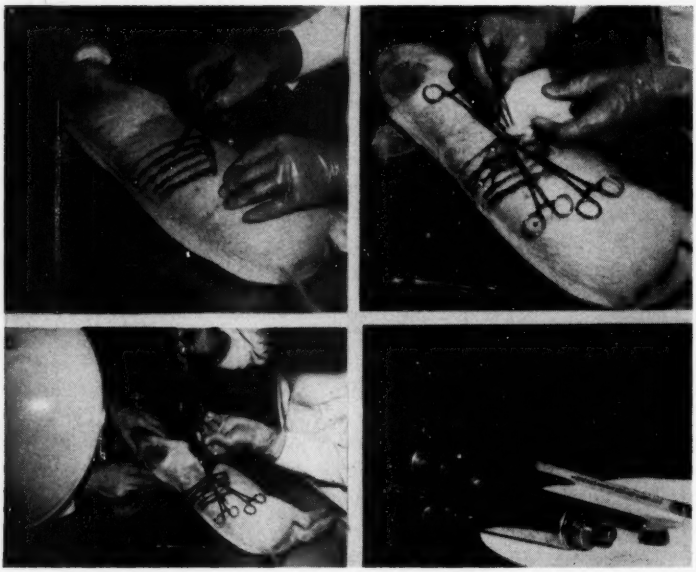

Figure 3. Liver biopsy of suckling pigs. A. anesthetized pigs and the point of incision. B. liver exposed. C. cannula and attached syringe obtaining liver sample. D. cannula, syringe, and typical sample obtained.

the eleventh intercostal space was made through the skin and musculature on the right side of the pig as shown in figure 3A.

The peritoneum was located, 2 hemostats were attached 1.0 cm. apart, and the peritoneum was pulled slightly out of the incision and incised about 2.0 cm. Two additional hemostats were attached to the peritoneum to facilitate suturing as shown in figure 3B.

The cannula and syringe shown in figures 1 and 3D were attached and locked. The cannula was then inserted into the incision and the point

placed perpendicular to the liver. Negative pressure was applied by withdrawing the plunger, illustrated in figure 3C. With only the aid of the instrument weight, the syringe and cannula were slowly turned two revolutions. The instrument weight was sufficient for the cannula to bore through the liver without damage to other tissue. One fatality occurred due to gall bladder damage.

The liver sample shown in figure 3D was deposited in physiological saline, washed free from blood, dried on filter paper, and immediately frozen for future analysis. Most samples obtained weighed between 0.7 and 1.0 gm. Only two sutures in each of the peritoneum, musculature, and skin were necessary. An antispetic powder was deposited in and on the incised area.

The entire operation can be completed in 20 minutes, depending of course on the skill of the operator. Also it is felt that this technique can be used routinely in many swine experiments. Pigs as small as 12 lb. have been biopsied using this technique.

Back-fat Biopsy in Swine

Many workers have removed back-fat samples from growing-fattening swine to measure the physical and chemical consistency of body fat. Most of these sampling techniques consist of incising the skin and "scooping out" a fat sample. The technique described below is a simple, rapid, and effective method of obtaining a more representative back-fat sample from live pigs.

The pig was restrained by means of a snout-loop. The hair was removed and a one-half inch incision was made through the skin by a quick thrust with a scalpel at the point of the shoulder. The trocar and cannula shown in figure 1 were inserted through the skin and pointed dorsally 80 degress to the vertical axis.

The trocar was removed and the syringe attached as shown in figure 4B and 4C, respectively. With a rotary motion, the cannula was inserted about 6 to 8 cm. Negative pressure was applied with the syringe and the cannula, syringe, and sample were removed. An even cylindrical sample as shown in figure 4D was obtained. Representative samples from both back-fat layers can be obtained with this method.

No infection has been observed as a result of biopsying. However, it is well to remove the sample, whenever possible, at such an angle as to prevent "blind pockets" from occurring.

Summary

A simple and rapid technique for liver biopsy of young pigs and beef cattle and back-fat biopsy of swine is described. All procedures were conducted with the same instruments adapted from a Record syringe and based on the aspiration principle.

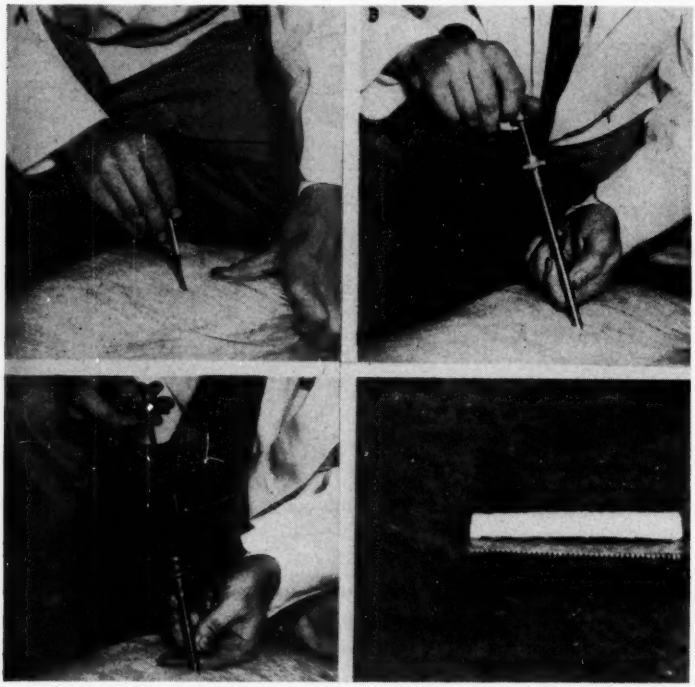

Figure 4. Back fat biopsy in swine. A. incision over point of shoulder. B. trocar being removed from the cannula. C. syringe attached to cannula. D. a typical sample obtained.

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A PORTABLE METABOLISM STALL FOR BEEF STEERS

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METABOLISM stalls have been used for many years by workers interested in quantitatively measuring the output of feces and/or urine from the various species of animals. The type of stall proposed by Nelson *et al.* (1954) is adapted to collections from ruminants. It entails many of the more recently developed features of other stalls.

The authors found that certain modifications of the stall described by Nelson *et al.* (1954) were necessary for the most efficient use and flexibility. Modifications that cause such stalls to lend themselves to handling a large number of animals were among those made. Further, most animals used at this station are far from docile; consequently, a stall providing for ease of loading the animals, combined with different stall dimensions, was constructed.

Description of Stall

The general assembly of the stall reported herein and those in use by Nelson *et al.* (1954) at Oklahoma are similar in many respects. Since the general description of this stall is similar, only the modifications and their respective merits will be discussed.

Figure 1A shows the metabolism stall with fecal pan in place. Increased height and side bracing of the stalls was necessary in order to provide strength for handling animals of different sizes and temperaments. Pipe instead of metal grid was used and proved to be equally effectve for efficient urine collection. In digestion studies, animals are required to live in stalls for a period of at least seven days. Comfort while in the stalls shortens the time necessary for the animal to adjust to new conditions. Rubber chute lining (not shown in photograph) one-fourth inch thick was placed on the floor of the stalls to increase comfort for the steers and to decrease the incidence of slipping and falling by the animals. Although Oklahoma workers reported the use of all-metal sliding feed hoppers, we have found that wood mounted on metal slides works equally as well. Also, rubber tubs inside the feed hoppers (Goodyear rubber troughs, No. 5) were used. Tare weights of the respective tubs were recorded and consequently increased the speed of feed intake

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measurements. The fecal pan is placed at floor level (figure 1A). Figures 1A and 1B show a sheet metal flashing from the floor of the stall to the fecal pan. This flashing serves two purposes: first, it directs some of the fecal material into the pan that might have otherwise been lost; and secondly, it holds the fecal pan in place.

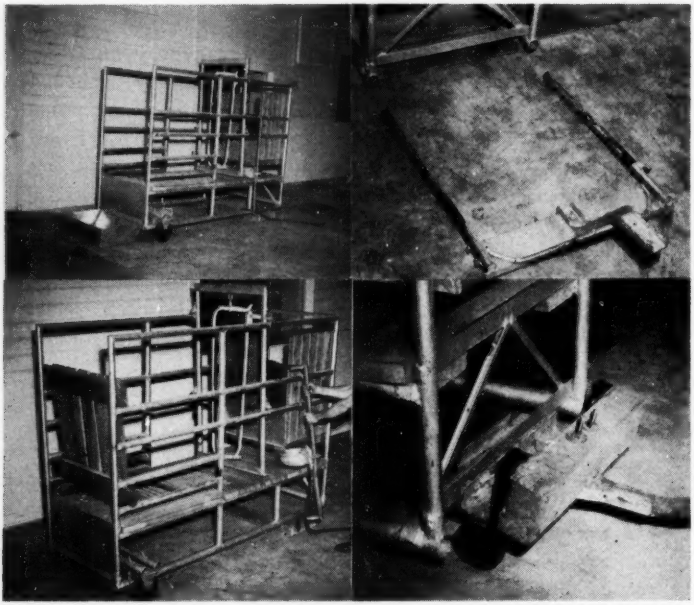

Figure 1A. Metabolism stall with fecal pan in place. 1B. Stall with wooden safety door and automatic waterer. 1C. Detachable hitch for transporting stall with a tractor. 1D. Front of stall and jack for hand movement.

The main features of the stall reported herein are shown in figure 1B. Two wheels were constructed on the back underneath side of the stall. Figure 1C illustrates a detachable hitch which attaches to the front of the stall. When the hitch is in place, the stall can be attached to a tractor and moved to a loading chute for exchanging animals. A jack with crazy wheels, shown in figure 1D, was constructed so that the stall can be moved or arranged, loaded or empty, without the aid of a tractor.

Automatic watering cups, as shown in figure 1B, were attached to a pipe with extended hooks so that cups could be disconnected easily.

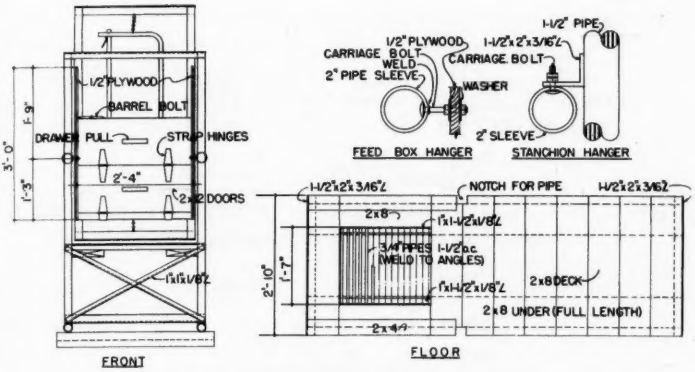

Figure 2. Floor and front details.

When all stalls were in place, each watering cup was connected by rubber hose to one main water outlet with a reduced pressure valve. This arrangement facilitated watercup disconnection before transportation of the stall.

The chute and corrals are about 250 feet from the metabolism room; moreover, the animals used in our studies had never been restrained. Consequently, a wooden removable door as shown in figure 1B was constructed so that the more nervous animals would not injure themselves by stepping back and down while in transit. Six animals can be

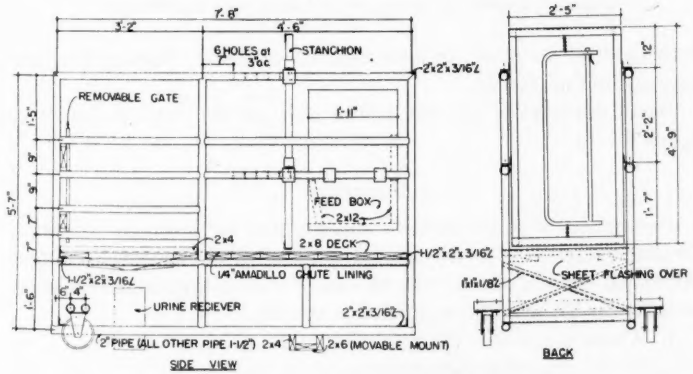

Figure 3. Details of the side and back.

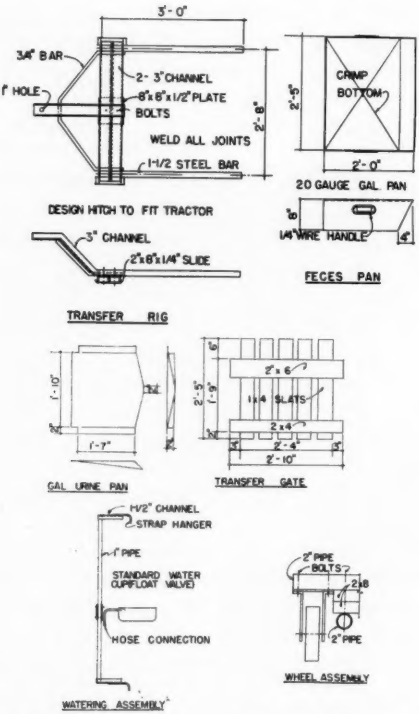

Figure 4. Details of various metabolism stall attachments.

exchanged for six others in approximately one hour, depending on the tameness of the animals.

Detail drawings of the metabolism stall are presented in figures 2, 3, and 4.

Summary

Description of a portable metabolism stall for steers is described. Desirable features of other reported stalls have been incorporated, plus additional features that provide for ease of stall movement and exchange of animals. These additional features include:

1. Wheels mounted to the metabolism stall.
2. Easily removable water system.

3. A jack with crazy wheels for hand movement and arrangement of stalls.
4. Removable hitch for a tractor.
5. Removable wooden rear door for use during steer transport.
6. One-fourth inch chute lining for comfort of animals.

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ANTIBIOTICS FOR THE PREVENTION OF BLOAT IN CATTLE GRAZING LADINO CLOVER [1]

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BLOAT is an economic problem wherever grassland agriculture or the improved pasture program is practiced. It is a particularly important problem in the delta regions of Mississippi, Arkansas and Louisiana, but is by no means confined to these areas. Death losses from bloat do not indicate fully the importance of this problem. Equally as important are the labor and expense of the stockman in trying to avoid bloat, and also the loss due to the restricted usage of certain legumes. In many cases the fear of bloat exceeds the problem that a farmer may have; but one cannot advocate complacency because even though cattle may bloat many times and not die, they certainly do die and sometimes in alarming numbers.

Many theories as to the cause of bloat have been proposed and as yet none are fully substantiated. Cole *et al.* (1945) and more recently Dougherty (1953) and Johns (1954) have reviewed and discussed these theories. It appears fairly certain that bloat is not caused by a single factor but probably by two or more factors.

An intensive study of the bloat problem has been underway for several years at this Station. A total of 1,575 cases of bloat were observed in 1954 and 1,643 cases were observed in 1955. As a result of these and earlier studies attention was focused toward rumen microorganism as a possible factor in the etiology of bloat. Therefore, it was decided to investigate some of the antibiotics for the prevention of bloat in cattle grazing Ladino clover.

Experimental Procedure

Paddocks containing almost a pure stand of Ladino clover were used in this study. Steers grazed the clover for 90 minutes, morning and afternoon. Grazing usually started at 7:30 a.m. and about 2:30 p.m. Under this system of grazing a steer could bloat twice daily. At the end of each grazing period the steers were checked for bloat and put in

[1] The antibiotics used were supplied by: Merck & Co., Rahway, N. J.; Lederle Laboratories, Pearl River, N. Y.: Chas. Pfizer & Co., Inc., Brooklyn, N. Y., and Commercial Solvents Corp., Terre Haute, Ind.

[2] Animal Husbandry Department, State College.

dry-lot with shade and water. Bloat was determined by visual observation, which was based upon direct and indirect ruminal pressure measurements. Doubtful cases were recorded as not bloated.

During the spring and summer of 1954 only steers a little over one year old and weighing 500 to 600 lb. were used. During the 1955 season both two-year-old steers (carried over from 1954) and new yearling steers were used. Only selected steers with a bloat history were used in this study. It is estimated that 90% or more of these steers would bloat at any given time.

The antibiotics were given orally in all cases in this study. Treatment was usually given between 11:30 a.m. and 1:00 p.m. Most of the time the antibiotic was given in a capsule with a balling gun, although in a few cases the antibiotic supplement was suspended in water and given as a drench. The period of protection was considered ended as soon as a steer had any visible distension, and steers were allowed to recover completely from the effects of previous treatment before being given antibiotics again.

The antibiotics tested for the prevention of bloat in this study were: chlortetracycline (Aureomycin), oxytetracycline (Terramycin), bacitracin, streptomycin, and penicillin. In preliminary studies as much as one gram of the antibiotic was given to a steer, however, due to extreme diarrhea this high dosage was not continued. An arbitrary dose of 300 mg. of each antibiotic was set as a limit.

Results

Chlortetracycline

In preliminary work, two steers were each given one gram of chlortetracycline by drench (126 gm. feed supplement containing 3.6 gm. per lb.). Both steers stopped bloating, one for 2.5 days and the other for 7 days. However, diarrhea was severe in both steers.

Smaller doses of chlortetracycline were then tried. Three steers were each given 100 mg. of chlortetracycline by capsule (1.82 gm. of crude chlortetracycline containing 25 gm. per lb.). Bloating was not inhibited.

Next two steers were each given 300 mg. of chlortetracycline by capsule. Bloating was not prevented.

Oxytetracycline

In a preliminary test, three steers were each given one gram of oxytetracycline by drench (45.4 gm. of crude oxytetracycline containing 10 gm. per lb.). All steers stopped bloating, one for 6 days, another for

10 days and the other for 7 days. Diarrhea was severe in all three steers.

Next, in a series of studies, levels of 50, 100, 150, 200 and 300 mg. of oxytetracycline were given to steers by capsule. Bloating was not prevented by any of these levels.

Bacitracin

Two steers were each given 300 mg. of bacitracin by capsule. Bloating was not prevented.

Streptomycin

Two steers were each given 300 mg. of streptomycin sulfate by capsule. Bloating was not prevented.

Penicillin

In a preliminary test, three steers were each given 1.0 gm. of procaine penicillin by capsule (5 gm. of a penicillin supplement containing 0.2 gm. of procaine penicillin per gram). All steers stopped bloating, one for 6 days, another for 7 days and the other for 10 days. Diarrhea was severe in all three steers.

In another trial two steers were each given 100 mg. of procaine penicillin by capsule. Both steers stopped bloating for 2.5 days each. No diarrhea was observed. In a third test two steers were each given 50 mg. of procaine penicillin by capsule. Both steers stopped bloating, one for 1.5 days and the other for 2.5 days. Again there appeared to be no undesirable effect. In the final test for 1954, four steers were each given 25 mg. of procaine penicillin by capsule. One steer continued to bloat, one stopped bloating for two days, and the other two steers stopped bloating for one day each.

Work on the value of penicillin for the prevention of bloat in steers grazing Ladino clover was continued in 1955. Two-year-old steers (carried over from 1954) and now weighing about 800 lb., and new yearling steers were used. The 1955 work included 14 separate trials with penicillin. A total of 57 doses of procaine or potassium penicillin were given to steers. The following observations were made:

1. Yearling steers responded to procaine penicillin as they had in 1954. Good protection was obtained with 25 mg. of procaine penicillin, and complete protection was obtained for 1.5 to 3 day periods with 50 mg. of procaine penicillin.

2. Two-year-old steers required higher levels of procaine penicillin for bloat prevention. Good protection was afforded by single doses of

50 mg. of procaine penicillin. Complete protection was obtained for 1- to 3-day periods by giving 75 mg. of procaine penicillin.

3. Pure potassium penicillin (in equivalent amounts) was equally as effective as procaine penicillin. There was an indication that potassium penicillin was more effective than procaine penicillin, but time did not permit further study of this observation.

Many rumen samples were taken by stomach tube from bloated steers, and in every case the ingesta was foamy in nature. Rumen samples taken 24 hours after penicillin treatment appeared to have less stable foam than rumen contents from bloated steers. Attempts to measure the amount of foam and its stability were not satisfactory.

Clover Feeding

All of the preceding work was conducted using the morning and afternoon grazing periods as previously discussed. No measure of forage consumption was possible except the observation that the steers grazed well during their allotted time. It is well known that large amounts of antibiotics affect the appetite of animals. The effect of small amounts of procaine penicillin upon clover consumption was investigated by clipping the clover and feeding it individually to steers. Previous work had shown that steers would bloat just as readily on fresh clipped clover as they would if grazed.

During the preliminary period four steers were put in individual pens and fed fresh clipped clover, morning and afternoon, to accustom them to this method of feeding. Then the four steers were fed all the clover they would eat during a 90-minute period morning and afternoon. During the morning average intake was 16.9 lb. of fresh clover (2.43 lb. dry), and in the afternoon 22.0 lb. of fresh clover (4.51 lb. dry). Late that afternoon all four steers were given 25 mg. of procaine penicillin by capsule. Feeding was continued the next day and in the morning the steers consumed 26.7 lb. of fresh clover (4.60 lb. dry). In the afternoon they consumed an average of 28.7 lb. of fresh clover (5.97 lb. dry). One steer bloated in the morning and three of the four steers bloated in the afternoon. These were two-year-old steers and it has already been stated that 25 mg. of procaine penicillin was not too effective on older steers.

The next feeding trial was conducted as before using four steers, except that 50 mg. of procaine penicillin was given. Before penicillin, the morning consumption of fresh clover averaged 16.4 lb. and the afternoon consumption was 22.4 lb. After penicillin, average clover consump-

tion was 22.5 lb. of fresh clover in the morning and 30.0 lb. in the afternoon. There were two old steers and two yearling steers in this group. One of the older steers bloated after the penicillin but the others did not.

Discussion

It has been stated that the antibiotics were usually given from 11:30 a.m. to 1:00 p.m. In almost every case treated steers bloated during the afternoon following treatment. Where treatment was effective bloating was prevented on the day following treatment and thereafter. This was true even when the 1.0 gm. doses of procaine penicillin, chlortetracycline, and oxytetracycline were given. Effective doses of penicillin given in the late afternoon were effective the next morning. Therefore it appears that a period of several hours or overnight is required before the antibiotic is effective in preventing bloat.

Steers did not usually bloat well for several days following the "protection period." Bloat was usually mild and irregular. In most cases, about a week was required for the steers to be bloating well enough to be used for another treatment. In a few cases, steers that were bloating well before treatment bloated so irregularly after penicillin treatment that they could not be used again.

A number of commercially available procaine penicillin products were used in this study. These products employed different carrier material as well as wide variations in the concentration of the procaine penicillin in the carrier. The nature and amount of the carrier did not appear to be a factor in these studies. However, antibacterial activity of the penicillin was definitely important. One penicillin product that was used had lost most of its activity and was ineffective in preventing bloat. This loss of activity was demonstrated by standard assay procedures.

Of specific interest is the fact that penicillin was effective at low concentrations, whereas the other antibiotics were not. Penicillin is known as a narrow-band antibiotic and is effective against relatively few types of organisms as compared to chlortetracycline or oxytetracycline. This would suggest that bloat on clover may be caused by a relatively specific type or types of microorganisms. Gall (see Marston, 1953) stated that there is a pointed rod type of organism usually present in numbers 100 times greater in animals that have bloated than in normal animals. This organism was a heavy gas producer.

Hungate *et al.* (1955), using a memometric method, reported that

the rate of rumen fermentation per gram of ingesta from bloated animals was significantly higher than from unbloated animals grazing on the same pasture. A stable foam was obtained in flask cultures containing ingesta from bloated animals and the possibility of slime production by microorganisms was considered.

Not a great deal is known about the effect of penicillin on rumen microorganisms. Beeson (1954) noted no ill effects when steers were fed 100 mg. of penicillin. Horn *et al.* (1955) fed varying amounts of penicillin to steers and noted no outward ill effects when as much as 800 mg. of penicillin was fed. However, the urine of steers fed 400 mg. of penicillin had a milky looking suspension with a yellowish cast. They reported that 100 mg. of penicillin did not appear to upset the rumen flora, whereas levels as high as 400 to 800 mg. of penicillin did change the flora. Working with the artificial rumen, Wasserman *et al.* (1952) reported that low levels of penicillin stimulated cellulose digestion. However, Huhtanen *et al.* (1954) and Hardie *et al.* (1953) concluded that penicillin inhibited cellulose digestion. Reid *et al.* (1954) have reviewed the general subject of antibiotics in the nutrition of ruminants.

An interesting observation was made by Tribe *et al.* (1954) while working with cobalt deficient sheep. The sheep did not respond to orally administered cobalt and it had been suggested that the rumen flora of such sheep might be so altered that they could not efficiently use the cobalt. These workers fed wethers a ration low in cobalt for five weeks, during which the wethers ate little feed and gained very little. Daily, oral administration of 50,000 units of sodium penicillin G was given for seven weeks and the wethers continued on the same ration. During the first three weeks after starting the penicillin there was little change, but during the next four weeks the wethers ate more feed and gained in weight. It was suggested that the penicillin so altered the flora of the rumen that the bacteria now flourishing were able to make better use of the cobalt in the diet.

Johns (1954) states that there appears to be at least two major groups of factors in the etiology of bloat: "a plant factor or factors usually associated with legumes, Crucifirae, and occasionally young ryegrass; and animals factors." The work presented in this paper would suggest "microorganism factors."

Summary

The oral administration of chlortetracycline, oxytetracycline, bacitracin, streptomycin and penicillin for the prevention of bloat in steers

grazing Ladino clover has been investigated. Penicillin was the only antibiotic studied that prevented bloat when a single dose of 300 mg. or less was given.

Single doses of 25 mg. of procaine penicillin gave good protection from bloat in yearling steers. Fifty mg. of procaine penicillin protected yearling steers in every case for 1.5- to 3-day periods. Older steers, weighing about 900 lb. required 50 to 75 mg. of procaine penicillin to prevent them from bloating. Clover consumption appeared to be slightly higher after penicillin treatment than before.

Potassium penicillin, in equivalent amounts, was equally as effective as procaine penicillin for the prevention of bloat. The nature of the procaine penicillin carrier and the concentration of the penicillin in the carrier appeared to have no effect.

Penicillin had to be given several hours or overnight before it was effective in preventing bloat in steers that were bloating before treatment.

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THE EFFECT OF PLANE OF NUTRITION ON THE MINERAL COMPOSITION OF BLOOD SERUM, LIVER, AND ON THE GROWTH OF BONE [1]

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THE analysis of body tissues can be useful in estimating the nutritional status of an animal with respect to a specific nutrient. However, the use of this technique is dependent on a knowledge of the effect of variables such as age, rate of growth, sex, level of nutrition, interrelations among nutrients, and of the "normal" variation of the tissue concentration of the particular nutrient. Such knowledge would be particularly valuable in studies of the micronutrient element nutrition of the bovine.

Materials and Methods

The growth and development of bone and the micronutrient element content of the blood serum and liver were studied in 60 Holstein bull calves at three levels of feed intake and at five ages. These animals were studied in two slaughter experiments performed in consecutive two-year periods. For each experiment, three-day-old grade calves were purchased at auction and put on experiment according to a completely randomized plan. The levels of feed intake provided 60 to 75 (low), 100 (medium) and 140 to 160 (high) percent of Morrison's total digestible nutrient higher allowances for growing dairy heifers (1950).

Calves on the low plane of nutrition were fed 175 lb. of whole milk the first 5 weeks, a maximum of 3.0 lb. of concentrates daily and poor quality timothy hay. Those on the medium plane received 370 lb. of whole milk during 7 weeks, up to 5.0 lb. concentrates and excellent quality hay, and the calves on the high plane received up to approximately 2,000 lb. of whole milk during the first 16 weeks, a maximum of 7.0 lb. of concentrates daily, excellent quality hay and a special supplement of vitamins A and D. The calves fed the high plane of nutrition also received a mineral supplement supplying 125 mg. iron, 50 mg. manganese, 10 mg. copper, and 4 mg. cobalt daily. The daily

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intakes of minerals calculated from analysis of composite food samples and feed consumption data for the first 48 weeks are shown in table 1. Further details of the experimental rations and techniques may be found in the thesis of Musgrave (1951) and that of Dunn (1952).

Samples of blood, liver and bone were obtained at slaughter ages of 16, 32, 48, 64 and 80 weeks. The blood sample was collected in nitric acid-cleaned, 250-ml. glass centrifuge bottles, permitted to clot, centrifuged, the serum removed and preserved frozen for later analysis. The caudate lobe of the liver was removed, weighed, cubed, frozen and stored in a plastic bag for analysis. The metacarpal and the first phalangeal bones of the third and fourth toe of the left foreleg were scraped free of tissue, weighed in air and in water, length and circumference measured, and then refrigerated (5° C.) for further processing.

TABLE 1. AVERAGE DAILY INTAKE OF MINERALS PER 100 LB. OF BODY WEIGHT

Nutrition plane	Ca	P	Mg	Fe	Mn	Cu	Co
	gm.	gm.	gm.	gm.	mg.	mg.	mg.
Low	6.5	4.3	2.3	0.13	67	21	0.9
Medium	8.8	5.0	2.8	0.13	61	22	1.1
High	9.9	7.3	4.4	0.25	175	28	2.3

The blood serums were analyzed for copper, molybdenum and manganese. These minerals plus iron and dry matter were determined on samples from the caudate lobe of the liver. The density of the fresh bone was calculated from its weight in air and in water, the moisture, ether-extract content determined and the dry fat-free weight calculated. Calcium, phosphorus, magnesium and manganese were determined on the first phalangeal bone of the fourth toe. The methods used were: copper, iron and phosphorus (Parks *et al.*, 1943); calcium and magnesium (Cheng and Bray, 1951); manganese (Gates and Ellis, 1947); molybdenum (Dick and Bingley, 1947). The blood serum and liver samples were wet ashed with a mixture of sulfuric, nitric and perchloric acids; the phalangeal bone was dry ashed at 600° C. All analyses were made in duplicate.

Results and Discussion

Analysis of variance (Snedecor, 1946) showed that age at slaughter and the ration fed had a highly significant ($P<0.01$) effect on the

following measures of bone development: fresh weight, dry weight, fat-free weight, length, circumference and density; and, on the ether extract, calcium, phosphorus, magnesium and manganese content of the fourth toe first phalanx. Further study of the data suggested a parabolic relation of the characteristic (Y) to body weight (W). These data were fitted to the general form $Y=aW^b$ by the method of least squares after logarithmic transformation. All of the regressions were highly significant ($P<0.01$). A typical regression line and its scatter plot is illustrated in figure 1. The calculated values of a and of b for

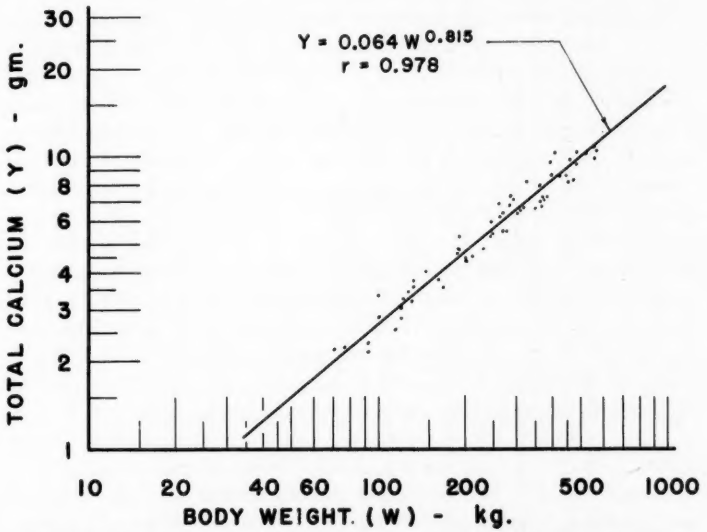

Figure 1. Regression of calcium content of fourth toe first phalanx on body weight.

the physical and chemical characteristics studied are presented in table 2 with the standard error of b and the correlation coefficient for each equation. This parabolic relation was also valid when calcium content was expressed as concentration in the dry, fat-free bone.

The density of the fourth toe first phalanx was a positive linear function of its calcium concentration on the dry, fat-free basis. The constants for this equation are given in table 2. The linear regression of the calcium concentration on density was highly significant ($P<0.01$).

The content of phosphorus (0.104 gm.$\pm$0.0007); of magnesium

TABLE 2. CALCULATED VALUES OF a AND b FROM THE EQUATION $Y=a\,W^b$ FOR THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF BONES OF THE LEFT FORE LEG OF BULL CALVES

Characteristic (Y)	Fourth toe, first phalanx				Third toe, first phalanx				Metacarpal			
	a	b	s_b[a]	r[b]	a	b	s_b	r	a	b	s_b	r
Fresh weight, gm.	2.29	0.552	±0.022	0.957	2.02	0.584	±0.073	0.957	20.8	0.486	±0.021	0.949
Dry, fat-free weight, gm.	0.392	0.752	±0.022	0.976	0.355	0.781	±0.023	0.975	3.43	0.707	±0.021	0.978
Length, cm.	2.63	0.168	±0.015	0.826	2.78	0.158	±0.015	0.806	10.90	0.119	±0.0074	0.901
Circumference, cm.	1.10	0.128	±0.013	0.802	1.03	0.145	±0.012	0.836	0.974	0.162	±0.012	0.875
Density	0.883	0.076	±0.006	0.869	0.872	0.0788	±0.0062	0.856	0.843	0.0919	±0.007	0.866
Ether-extract, gm.	0.247	0.690	±0.048	0.883	0.276	0.676	±0.047	0.883	3.589	0.522	±0.067	0.742
Total calcium, gm.	0.0635	0.815	±0.023	0.978								
Total phosphorus, gm.	0.392	0.759	±0.025	0.970								
Total magnesium, gm.	0.0020	0.695	±0.119	0.638								
Total manganese, mcg.	0.0665	1.011	±0.086	0.714								
Calcium per gm. dry, fat-free bone, gm.	0.165	0.059	±0.0094	0.634								
Calcium per gm. dry fat-free bone on density (Y=a+bD)	0.0868	0.105	±0.038	0.610								

[a] Standard error of b.
[b] Correlation coefficient.

(0.0042 gm.±0.0002); and the calcium to phosphorus ratio (2.21 gm.±0.148) per gram of dry, fat-free bone of the fourth toe first phalanx was not affected by the experimental treatments: neither were the moisture contents of the first phalangeal bones (12.1 gm.±1.98, third toe; 11.4 gm.±1.98, fourth toe) nor of the metacarpal 65.4± 14.21).

At all slaughter ages the general observation was made that the medial articulate area of the volar surface of the metacarpus had an eroded area. This erosion varied in depth and area covered without apparent relation to age or size of the animal. This interruption in the articular surface is considered to be of normal occurrence in the bovine.

The relation of the physical and chemical characteristics of bone to body weight reported here extends Brody's (1945) observations on linear growth, form and function to the growth and development of specific bones under well defined nutritional regimes. In calves fed a nutritionally balanced ration, the effect of varying the level of total digestible nutrient intake was to regulate the growth and development of bone to the rate of increase in body weight. In terms of chronological age, the effect of the plane of nutrition was to accelerate or retard bone development without affecting its physical or chemical integrity. In terms of physiological development, the bone of calves at the same body weight had similar physical and chemical characteristics regardless of the plane of nutrition fed or the chronological age of the animal. The extent of recovery from growth retardation cannot be predicted from these results, although normal home development continued to age 80 weeks in the bulls fed at the low plane of nutrition.

The copper, molybdenum, and manganese content of blood serum and liver, and the iron and water content of the liver are shown in table 3. The mean values are tabulated for age, plane of nutrition, and for experiments A and B when such a difference between experiments existed. The standard error of the mean as calculated from the mean square for within animal variance with 30 degrees of freedom, the coefficient of variation for the overall mean and the range of the individual values are given.

The blood serum level of copper, molybdenum and manganese did not differ with age or experiment. Plane of nutrition has a highly significant ($P<0.01$) effect on blood serum copper but had no influence on the concentration of molybdenum or manganese in the serum.

The serum copper content was 20% higher in the animals fed at the high plane of nutrition than in those fed at the medium or low levels.

TABLE 3. CONCENTRATION OF COPPER, MOLYBDENUM AND MANGANESE IN BLOOD SERUM AND LIVER, AND LIVER IRON

				Liver							
	Blood serum			Cu		Mo		Mn	Fe		Water
Age	Cu	Mo	Mn	A[a]	B[a]	A	B	A & B	A	B	A & B
Weeks	mcg. per 100 ml.			mcg. per gm. dry matter							%
16	60	4.4	0.57	542	502	5.1	3.9	9.3	194	218	70.0
32	63	5.0	0.46	318	395	5.5	4.3	8.2	137	266	69.6
48	56	4.1	0.55	250	294	5.1	5.0	8.9	138	172	65.3
64	67	6.2	0.50	128	368	4.9	4.2	9.7	198	144	65.0
80	56	5.2	0.84	148	257	4.5	4.5	8.5	171	203	56.3
St'd error	±5.78	±0.88	±0.17	±27.5		±0.35		±0.55	±20.8		±2.50
				Plane of nutrition							
Low	54	5.1	0.45	272	356	4.9	3.9	8.9	150	207	66.1
Medium	57	4.4	0.61	235	298	5.1	4.9	8.8	184	202	65.3
High	70	5.4	0.69	325	435	5.2	4.4	9.0	170	193	64.2
St'd error	±4.48	±0.69	±0.13	±21.3		±0.27		±0.41	±15.1		±1.94
Range	30–110	1.0–13.5	0.10–3.0	104–672		1.8–8.0		2.6–16.2	106–396		43.4–74.0
Coefficient of var. %	23.5	43.8	71.3	29.7		26.2		15.0	27.7		

[a] A and B refer to data obtained in the two experiments.

The water content of the liver was affected ($P<0.01$) by the age of the calf. As the animal aged, the water content of the liver decreased progressively with a relative difference of 24% in water content between age 16 and 80 weeks.

The age of the animal, the level of feed intake and the experiment had a highly significant ($P<0.01$) effect on the concentration of copper in the dry matter of the liver, and the interaction between age and experiment was significant ($P<0.05$). Application of the Duncan test (1951) indicated that the copper content of the liver decreased progressively from 16 to 48 weeks of age with no further reduction to age 80 weeks; that the calves fed at the high rate of TDN had more copper stored in their livers than those fed at the medium or low rate; that the copper content was lower in the livers of the animals from the first experiment than those from the second experiment; and that the decrease with age of the calf was greater in the first experiment than in the second.

The molybdenum concentration in the liver dry matter was different ($P<0.05$) between experiments, but the age of the calf or the level of feed intake did not influence this concentration. The age of slaughter, plane of nutrition and experiment did not affect the manganese content of the liver. The interaction between level of feed intake and age on manganese concentration was highly significant ($P<0.01$). The level of iron in the liver dry matter did not differ with age or rate of feed intake, but did differ ($P<0.05$) with the experiment. The interaction between experiment and age was significant ($P<0.05$).

The blood serum copper levels of bull calves observed in this study are lower than those levels reported by Matrone *et al.* (1947) for heifers of similar ages, but fall within the range of values reported in the literature and tabulated by Matrone *et al.* The variability in the copper levels of bovine serum reported in the literature for healthy animals may be accounted for in part by the influence of age, of level of copper consumption and possibility of sex. The involvement of sulfur and molybdenum in copper metabolism recently reported by Dick (1954) may be another factor influencing serum copper levels in animals from different locations.

The higher levels of copper in the blood serum and livers in those animals fed at the high plane of nutrition than in those fed at the medium or low levels apparently reflected the increased rate of copper consumption at the high plane of nutrition. An increase in the rate of copper consumption from 21 to 28 mg. copper per 100 lb. body weight

daily resulted in larger body stores of this element, but whether or not these increased stores of copper are nutritionally beneficial or desirable has not been determined in this study.

The differences in mineral composition of the livers between the two experiments cannot be explained on the basis of any known variation in the animals or rations used. The progessive decline in liver copper concentration from age 16 to 48 weeks probably represents a gradual adjustment from the high copper stores at birth (Bourne and Kidder, 1953).

Summary

In a slaughter experiment with Holstein bull calves, the effect of age and plane of nutrition was studied on the growth and development of bone and the micronutrient element content of blood serum and liver tissue. The calves were provided with 60 to 75, 100 and 140 to 160% of Morrison's higher total digestible nutrient allowances for growing dairy heifers; and were slaughtered at 16, 32, 48, 60 and 80 weeks of age. A parabolic relation of the physical and chemical characteristics of bone to body weight was observed. Bone from calves of the same body weight were in the same stage of development regardless of age or plane of nutrition fed. Normal bone growth continued in the underfed animals to the final slaughter age.

The blood serum level of copper (60.0 ± 5.8 mcg. per 100 ml), molybdenum (5.0 ± 0.88 mcg. per 100 ml.) and manganese (0.59 ± 0.17 mcg. per 100 ml.) did not change with age. Serum copper levels increased with a higher rate of copper consumption. The concentration of iron (184 ± 20.8 mcg. per gm.), molybdenum (4.7 ± 0.35 mcg. per gm.) and manganese (8.9 ± 0.55 mcg. per gm.) in the liver dry matter was not affected by the age of the calf or plane of nutrition. The copper concentration in the liver dry matter decreased progressively with age up to 48 weeks of age, and increased at the higher level of copper consumption. The water content of the liver decreased progressively as the animal aged.

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RELATIONSHIPS BETWEEN LEVEL OF PROTEIN, MOLASSES, TRACE MINERALS AND QUALITY OF HAY IN RATIONS FOR FATTENING CATTLE

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GUILBERT, Gerlaugh and Madsen (1950) have discussed the discrepancy which exists between the amount of digestible protein required by fattening cattle when calculated by the factorial procedure (Mitchell, 1929; Blaxter and Mitchell, 1948) and the amount found to be necessary in feeding trials for optimum feed consumption and rate of gain (Morrison, 1948). The smaller amount of protein, as determined by the factorial procedure, apparently has not been adequate for greatest returns when a high energy, fattening ration is fed.

Recent studies in swine nutrition have shown that the amount of protein required by growing, fattening swine may be decreased when their ration is adequately fortified with B vitamins (Robison, 1952, 1953; Hoefer *et al.*, 1952; Wallace *et al.*, 1954 and Burnside *et al.*, 1954).

Previous investigations conducted at this Station (Klosterman *et al.*, 1953) have shown that a ration for fattening cattle composed of ground ear corn, soybean oil meal and poor quality timothy hay was deficient in trace minerals. In one experiment the addition of one pound of cane molasses per head daily significantly increased the rate of gain of cattle fed a ration sub-optimal in protein. These experiments have been continued to: (1) further study the value of hay quality in rations for fattening cattle, (2) determine if any beneficial effect would be realized from feeding molasses when trace minerals were included in the ration, (3) further study the protein requirements of fattening calves, and (4) study any relationships that might exist between level of protein, molasses and quality of hay.

Procedure

Two experiments have been conducted with Hereford steer calves fattened in dry lot. In the first experiment, 1952–1953, twelve steers were fed each ration for 196 days and in the second, 1953–1954, seven steers were fed each ration for 238 days. The calves weighed approxi-

mately 450 lb. at the start of the experiments and were full fed ground ear corn and allowed free access to salt and a mineral mixture of two parts steamed bone meal, two parts ground limestone, and one part salt.

The hay fed in the first experiment, and that designated as poor quality in the second experiment, was late-cut timothy which was intentionally allowed to weather prior to baling. The good quality hay fed in the second experiment was early cut, light legume mixed timothy harvested without weathering.

The protein supplement fed in the first experiment was a mixture of solvent extracted soybean oil meal and feed grade urea with approximately equal amounts of nitrogen being supplied by each source. Solvent extracted soybean oil meal was fed in the second experiment. In the rations where fed, blackstrap, cane molasses was used.

The trace minerals fed in the 1952–1953 experiment were mixed with finely ground ear corn in such amounts that 1 lb. of the mixture supplied the following amounts of mineral elements per steer daily: iron, 800 mg.; manganese, 200 mg.; copper, 25 mg.; zinc, 14 mg.; and cobalt, 0.5 mg. In the second experiment, a commercial trace mineralized salt was fed with all rations.

In addition to the two feeding experiments, one digestion experiment was conducted to study the effect of feeding molasses upon the digestibility of dry matter and protein when two levels of protein were fed. Steers which had been on a full feed of ground ear corn were used for these studies. Four steers were fed 1.5 lb. and four, 0.75 lb. soybean oil meal per head daily. Two of each group were fed 1.0 lb. molasses per head per day. All steers were fed good quality mixed hay and a full feed of ground ear corn. After one collection was completed the rations were reversed relative to molasses feeding and a second collection was made. In these experiments the chromic oxide marker technique was used.

Results

The results obtained in the first experiment, where various supplements were fed with poor quality hay and ground ear corn, are presented in table 1. Statistical analysis of the gains made by these cattle showed a highly significant difference between the two levels of protein fed and also between treatments. The cattle fed 1.5 lb. of soybean oil meal equivalent gained more rapidly than those fed 0.75 lb. soybean oil meal equivalent. The addition of molasses or trace minerals significantly increased gains at both levels of protein.

When neither molasses nor trace minerals were fed there was very little difference in rate of gain between cattle fed the two levels of protein. The addition of trace minerals significantly increased the rate of gain at both levels of protein. However, the increase was significantly more at the higher protein level. These results indicate that the lower protein ration was deficient in both protein and trace minerals and that the addition of protein was of little benefit without the addition of trace minerals.

TABLE 1. SUPPLEMENTS TO POOR QUALITY HAY FOR FATTENING CATTLE 1952–1953 EXPERIMENT

	Control	One pound molasses	Trace minerals
1.5 lb. soybean oil meal equivalent per head daily			
Average daily gain, lb.	1.76	2.12	2.18
Average daily ration, lb.:			
Concentrates[a]	11.9	14.0	13.0
Hay	2.3	2.3	2.1
Feed per cwt. gain, lb.:			
Concentrates[a]	679.0	657.0	596.0
Hay	133.0	107.0	98.0
0.75 lb. soybean oil meal equivalent per head daily			
Average daily gain, lb.	1.72	2.07	1.90
Average daily ration, lb.:			
Concentrates[a]	11.4	13.9	12.6
Hay	2.1	2.1	2.3
Feed per cwt. gain, lb.:			
Concentrates[a]	761.0	674.0	675.0
Hay	124.0	103.0	123.0

[a] Includes ground ear corn, soybean oil meal, urea, minerals and molasses where fed.

There was a higly significant increase in rate of gain at both levels of protein due to the addition of molasses. When molasses was fed, there was no significant difference between the two levels of protein. These comparisons indicate that the molasses supplied the necessary trace minerals and also had an apparent sparing effect upon the protein requirement.

The feeding of either trace minerals or molasses increased the daily corn consumption in approximately equal amounts and the cattle fed 1 lb. of molasses ate it in addition to the corn. With the lower protein ration the cattle fed molasses required slightly less feed per

unit of gain than those fed trace minerals. The reverse was true when fed with the higher protein ration.

The second feeding experiment was conducted to study the relationships between molasses, quality of hay and level of protein when trace minerals were fed with all rations. The results of this experiment are presented in table 2.

Statistical analysis of the average daily gains obtained in this experiment showed that the differences between good and poor hay, levels of protein and the interaction between quality of hay and level of protein were all significant at the 1% level. The difference between the two hays became greater as the amount of protein in the ration was decreased. There was no significant difference between the two hays when fed with 1.5 lb. of soybean oil meal per head daily but the differences were both highly significant at the two lower levels of protein. With regard to level of protein, there was no significant difference in rate of gain between steers fed 1.5 lb. and 0.75 lb. of soybean oil meal with the good quality hay. All other comparisons of levels of protein were significant at the 1% level.

A part of the difference between good and poor quality hay and the interaction between hay quality and level of protein can be explained by the difference in protein content and digestibility of the two hays and the difference in amount of hay consumed. The good quality hay contained 12.0% and the poor hay, 6.9% crude protein. Assuming digestion coefficients of 60 and 40% for the protein of the good and poor hays, respectively, the difference in digestible protein consumed from the two hays would be approximately equal to the amount of digestible protein in 0.3 lb. of soybean oil meal. However, it will be noted in table 2 that the steers fed good hay gained at much the same rate as those fed poor hay and 0.75 lb. more soybean oil meal. In the four instances where this comparison can be made, the differences are not statistically significant. These results indicate that a part of the difference between the two hays was due to something other than their difference in protein content. Neither does this difference appear to be due to trace minerals, since a trace mineralized salt was fed with all rations.

The feeding of 1 lb. of molasses per head daily did not significantly improve the rate of gain of steers fed either the good or poor quality hay rations when trace minerals were included in both. Neither was the interaction between molasses and level of protein significant. Although this interaction was not significant in this experiment, the

TABLE 2. RELATIONSHIPS BETWEEN LEVEL OF PROTEIN, MOLASSES AND QUALITY OF HAY IN RATIONS FOR FATTENING CATTLE

1953–1954 Experiment	Good hay		Poor hay	
	No molasses	One pound molasses	No molasses	One pound molasses
1.5 lb. soybean oil meal per head daily				
Digestible protein per head daily, lb.	1.50	1.50	1.38	1.38
Average daily gain, lb.	2.12	2.14	2.03	2.12
Average daily ration, lb.:				
Concentrates[a]	14.4	15.3	14.3	15.4
Hay	2.5	2.5	2.0	2.0
Feed per cwt. gain, lb.:				
Concentrates[a]	676.0	713.0	704.0	723.0
Hay	116.0	114.0	100.0	96.0
Dressing percentage	61.8	61.5	61.4	60.3
Carcass grade[b]	0.90	0.70	1.17	1.21
0.75 lb. soybean oil meal per head daily				
Digestible protein per head daily, lb.	1.22	1.25	1.08	1.08
Average daily gain, lb.	1.98	2.19	1.79	1.92
Average daily ration, lb.:				
Concentrates[a]	14.2	15.4	13.6	14.8
Hay	2.5	2.5	2.0	2.0
Feed per cwt. gain, lb.:				
Concentrates[a]	712.0	700.0	761.0	769.0
Hay	124.0	112.0	113.0	106.0
Dressing percentage	59.5	61.5	58.5	59.1
Carcass grade[b]	1.37	0.77	1.47	1.46
No soybean oil meal				
Digestible protein per head daily, lb.	0.92	0.91	0.72	0.70
Average daily gain, lb.	1.83	1.82	1.33	1.29
Average daily ration, lb.:				
Concentrates[a]	13.2	14.0	11.8	12.6
Hay	2.5	2.5	2.0	2.0
Feed per cwt. gain, lb.:				
Concentrates[a]	727.0	767.0	891.0	975.0
Hay	134.0	135.0	154.0	157.0
Dressing percentage	59.7	59.2		
Carcass grade[b]	1.51	1.57	2.54[c]	2.57[c]

[a] Includes ground ear corn, minerals and soybean oil meal and/or molasses where fed.
[b] Grade factors: Prime, 0.4, 0.7; choice, 1.0, 1.4, 1.7; good, 2.0, 2.4, 2.7.
[c] Live slaughter grade given for these two lots as these steers were not slaughtered at the end of the experiment.

molasses tended to increase the daily rate of gain of cattle fed 0.75 lb. of soybean oil meal (0.21 lb. with good hay and 0.13 lb. with poor hay). This is in agreement with the results obtained in the first experiment and in a previous publication (Klosterman *et al.*, 1953) where feeding molasses significantly increased growth rate of cattle fed 0.75 lb. soybean oil meal. In the previous publication and in this experiment molasses was of no benefit to gains when 1.5 lb. of soybean oil meal was fed. Neither was it of any benefit when no soybean oil meal was fed.

Statistical analysis of the carcass grades obtained in the 1953–1954 experiment showed differences which in general agreed with the differences which occurred in average daily gains.

TABLE 3. APPARENT DIGESTIBILITY OF RATIONS FOR FATTENING CATTLE AS AFFECTED BY MOLASSES AND AMOUNT OF SOYBEAN OIL MEAL INCLUDED IN THE RATION

Soybean oil meal per head daily	No molasses	One pound molasses	Average
	Digestibility of protein, %		
1.5	57.6	57.9	57.8
0.75	53.0	47.7	50.4
Average	55.3	52.8	
	Digestibility of dry matter, %		
1.5	62.8	67.2	65.0
0.75	63.6	61.0	62.3
Average	63.2	64.1	

The results obtained from the digestion experiment are presented in table 3. It will be noted that the digestibility of protein tended to be lower in the lower protein rations and also that, on the average, the digestibility of dry matter was slightly lower. With the number of observations made, however, none of the differences proved statistically significant.

Discussion

The results presented in this paper are in agreement with a previous publication from this Station (Klosterman *et al.*, 1953) in which a ration for fattening cattle of ground ear corn, soybean oil meal and poor quality timothy hay was significantly improved by the addition of trace minerals or cane molasses. In the present experiments molasses was of no additional benefit when fed with a poor quality hay

ration which included trace minerals. These results and those presented in the previous publication also indicate that good quality mixed hay contains some beneficial factor which cannot be completely accounted for by its protein or trace mineral content.

In four experiments which have been made, molasses appears to have some beneficial effect on rate of gain, when fed with a ration sub-optimal in protein. The addition of 1 lb. of molasses to a ration containing 0.75 lb. of soybean oil meal has consistently increased the rate of gain. This effect is difficult to explain in light of the fact that molasses generally reduces the digestibility of protein (Briggs and Heller, 1940, 1945; Bohman *et al.*, 1954). It should be noted in the present experiments that cattle fed molasses have consumed as much if not more ground ear corn than those fed no molasses and in addition have consumed the 1 lb. of molasses. It may be that the palatability of molasses counteracts the reduction in appetite which accompanies a ration sub-optimal in protein. This was not true, however, when a ration markedly deficient in protein was fed. Molasses has long been used to increase consumption of poor quality roughage and other unpalatable feeds. Plice, (1952), reported that sweetening agents, other than sugar, stimulated the consumption of otherwise unpalatable forages. However, in a limited observation made at this Station (unpublished data), sprinkling a solution of calcium cyclamate "Sucaryl" on ground ear corn did not stimulate its consumption when the ration was sub-optimal in protein.

Summary

A ration of ground ear corn, soybean oil meal and poor quality timothy hay was significantly improved by the addition of trace minerals or molasses when fed to fattening steer calves. Molasses was of no apparent benefit when added to a ration which included trace minerals or good quality mixed hay.

There were highly significant differences in rates of gain of steers fed various amounts of protein. The amount of protein in the ration was of much greater importance when fed with poor quality timothy hay than with good quality mixed hay. Results obtained indicate the presence of some beneficial factor in good quality hay other than protein or trace minerals.

Cane molasses seemed to have some sparing effect on protein requirement when a ration sub-opitmal in protein was fed but not with a

ration markedly deficient in protein. This effect was a result of increased feed consumption and not improved feed utilization.

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THE RELATIONSHIP OF FATNESS IN YEARLING STEERS TO JUICINESS AND TENDERNESS OF BROILED AND BRAISED STEAKS [1, 2]

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BEEF which rates dependably high in juiciness and tenderness is desired by consumers. The belief that such qualities are obtained by fattening an animal has been expressed by Armsby (1908, 1917), Bull (1916), Hall (1910), Helser (1929), and Henry and Morrison (1916). However, proving that tenderness and juiciness of beef are increased to a marked degree by fattening has been difficult. Although the differences in tenderness and juiciness in many studies have been in favor of the fatter animals rather consistently, the actual differences have been small (Black *et al.*, 1931; Hankins and Ellis 1939; Barbella *et al.*, 1939; Wanderstock and Miller 1948; Husaini *et al.*, 1950). In other studies no significant differences were found between animals differing in fatness (Black *et al.*, 1940; Husaini *et al.*, 1950).

In previous studies juiciness and tenderness have been tested on standing rib roasts from 9–10–11 ribs cooked rare under standard conditions as recommended by Cooperative Meat Investigations. The cooking time for roasts is long in comparison with that needed for steaks. This longer cooking time may minimize differences inherent in the meat. Meat from only one position in the carcass has been used in previous tests but it is not known whether other positions in the carcass might be more responsive to differences in fatness. The purpose of this study was to determine whether fatness influences the eating quality of loin and bottom round steaks when cooked by broiling and braising.

Experimental Procedure

A total of 47 Hereford and 50 Brahman × Hereford steers out of Hereford cows were placed on the test rations about 2 weeks after the

[1] The meat used was from animals raised at Substantion 23, McGregor, Texas under a project which is cooperative with the U. S. Department of Agriculture and is a part of S–10 Southern Regional Beef Cattle Breeding Project.

[2] The authors wish to acknowledge the assistance of Dr. M. J. Garber, Department of Genetics, for his help with the statistical designs and R. L. Smith, Statistical Laboratory, for making the statistical calculations.

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calves were weaned in November 1953. A 14-day adjustment period preceded the 140-day rate of gain test. Two rations were fed. The "growing" ration contained 35% concentrates and the "fattening" ration 65% concentrates.

From the 97 steers on the test, 38 were selected for slaughter. Four of the progeny from each of five Brahman bulls gave a total of 20 crossbred steers and four from each of three Hereford bulls and six from another gave a total of 18 Hereford steers. The steers were selected at random within the sire groups and were evenly divided between the two rations. The steers were kept on the experimental rations until slaughter, 15 to 18 days after the gain test was completed. At slaughter in May 1954, the steers averaged 14 months of age, and ranged from 13 to 15 months. The Herefords averaged 743.5 lb. at slaughter and the crossbreds 782.2 lb. A report of the evaluation tests for gaining ability has been published elsewhere (Warwick and Cartwright, 1955).

The 38 steers were brought from McGregor to College Station and slaughtered in the meats laboratory of the Animal Husbandry Department. The carcasses were graded by a government grader. Eight of them graded U. S. Choice, twenty-five U. S. Good, and five U. S Commercial. The relation of carcass grade to eating quality of these steers will be reported in a subsequent paper. The carcasses were stored at about 36° F. for either 7 or 8 days. Six steaks three fourths inch thick from each side of each of the 38 carcasses were obtained from the short loin and bottom round for chemical analyses and for palatability and shear tests. The bone was removed from the loin steaks to facilitate storage in the freezer and to help prevent cupping during cooking. The individual steaks were wrapped and numbered. Loin steaks were numbered from the anterior end, bottom round steaks from the dorsal end. They were frozen at about minus 20° F. and stored at about 0° F. until the tests could be made.

Measures of Fatness

Fatness was recorded in three ways: by physical separations, by estimations of marbling and by determinations of ether extract. The physical separations were made on the left 9–10–11 rib cut and the resulting percentage of separable fat was used in the formula developed by Hankins and Ellis (1946) to estimate the separable fat in the carcass. Marbling is the presence of fat in tiny flecks within the muscle, and the more tiny flecks there are the more tender and juicy the meat

is thought to be. When a beef carcass is graded, marbling is given major consideration. Marbling at the 12th rib was recorded as the estimated area of visible fat expressed as an estimated percentage of the total area of the eye muscle. Ether extract was determined in three trimmed muscles. The percentage of ether extract within a muscle is thought to be a chemical measure of marbling. Other data on these carcasses have been published elsewhere (Butler *et al.*, 1956).

Analyses for moisture and ether extract were made on the raw meat of longissimus dorsi (ribeye) muscle in the left 9–10–11 rib cut, on longissimus dorsi in loin, and on biceps femoris in bottom round. The steaks used for the raw samples were: loin 1 and 6 left, 2 and 5 right; bottom round, 1 and 5 left, 2 and 6 right. Analyses were made on the meat after the outside edge of the muscle had been trimmed away so that adhering fat would not influence the results. The percentage ether extract was reported on the dry basis. The analytical methods were essentially those described in A.O.A.C.

Palatability and Shear Force Tests

Steaks 3 and 4 on each side from each cut from each animal were used for palatability and shearing tests. Paired cuts from the right and left sides of the same animal were cooked—one by broiling and its pair mate by braising. The steaks were cooked well done—no pink color. The broiling and braising methods have been described in more detail by Cover and Shrode (1955). The broiler was preheated to 200° C. (392° F.). The steak was browned well on one side (approximately 25 minutes, turned and browned on the other side (approximately 10 minutes). Steak thermometers were not successful in the steaks but a table of cooking times based on weight and shape was devised for these thin steaks. Braised steaks were browned under standardized conditions and then cooked in steam for 45 minutes. The steak temperature reached 100° C. in about 20 minutes and was held there for about 25 minutes (Cover and Shrode, 1955). The entire steak was cooked but after cooking an individual muscle was used for scoring and shearing tests. The muscles used were: longissimus dorsi in loin and biceps femoris in bottom round.

Shear force values were obtained with a Warner-Bratzler shearing device. Four cores one half inch in diameter were obtained, two at one end of the steak and two at the other. The cores were so thin that only one shear from each core was obtained, making a total of 4 shears from each steak. Palatability scores for juiciness and tenderness were

obtained from a panel of five judges. The composition of the judging committee was uniform throughout the test. The samples for the judges were small pieces of meat—not an entire steak. They were narrow strips taken from the area in the central portion of the steak and between the two sets of cores. Each of these samples from a broiled steak was paired with a sample from a similar position in the braised steak of the pair. The judges used a score card of adjectives weighted from zero to ten in which 10 is excellent, 9 very good, 7 good, 5 medium, 3 poor, 1 very poor, and 0 unacceptable.

Results and Discussion

The percentages of fat obtained by the various measures are given in table 1. To determine how closely the various methods of measuring fatness agreed, simple correlations were calculated. The coefficients are given in table 2. All were highly significant. The correlation between the two estimated measures of fatness is high (0.784) as is the correlation between ether extracts (0.832 and 0.742). But those between estimated marbling and the ether extracts are lower (0.597, 0.654 and 0.668) and those between estimated fat in the carcass and the ether extracts are the lowest of all (0.515, 0.556 and 0.471). It may be noted that ether extract measures not only the fat seen as flecks of marbling but also that which occurs in deposits along seams of heavy connective tissue. This would not be called marbling. Then, too, the ether extract values may be affected by deposits of fat too small to be seen as estimated marbling and by small amounts of ether soluble substances which are not true fats. It may be that one or more of these factors have little if any relation to estimated fat in carcass or to estimated marbling, and their relationship to juiciness and tenderness is unknown.

The average scores for juiciness and tenderness and the average shear force values are given in table 3. Other data on these steaks have been reported by Cover and Smith (1956). Correlation coefficients for each of the ways of reporting fatness with the juiciness and the tenderness of the cooked meat are given in table 4. Comparison within each factor of eating quality may indicate whether all the cuts and methods of cooking are of equal value in showing an association with fatness. Juiciness scores seemed to be more closely correlated with fatness in broiled loin steaks than in braised loin or in broiled or braised bottom round. Tenderness scores seemed more closely correlated with fatness in bottom round than in loin. Shear force values and

TABLE 1. FATNESS AS MEASURED IN DIFFERENT WAYS

Measures of fatness	Percentage of fat	
	Mean (S.D.)	Range
Estimated fat in carcass[a]	28.4 (4.3)	19.5—36.9
Estimated marbling[b]	6.7 (3.0)	1.0—12.0
Ether extract 9–10–11 ribeye[c]	8.8 (3.1)	3.8—15.7
Ether extract L. dorsi in loin[c]	8.6 (2.9)	4.2—17.2
Ether extract B. femoris in bottom round[c]	9.6 (2.9)	4.6—15.8

[a] Based on formula of Hankins and Howe.
[b] Estimated percent fat in estimated area of ribeye at 12th rib.
[c] Dry basis.

fatness seemed most closely correlated in braised bottom round. Apparently conclusions based on results obtained from loin steaks were not applicable to bottom round steaks. This raises the important question of whether the tenderness or juiciness from one or two cuts can be used with any assurance for generalizing about the meat from the other cuts.

Studies on the relationship between fatness and juiciness and between fatness and tenderness have been conducted under Cooperative Meat Investigations and have been reported in the literature. In these studies the left 9–10–11 standing rib cut was roasted rare by dry heat under standarized conditions. Hankins and Ellis (1939) used ether extract

TABLE 2. SIMPLE CORRELATIONS FOR THE VARIOUS WAYS OF REPORTING FATNESS

Sources of variation	Coefficients of Correlation	Percentage of variation accounted for
	r	$r^2 \times 100$
Estimated fat in carcass versus		
Estimated marbling	0.784***	61
Ether extract of 9–10–11 ribeye	0.515**	27
Ether extract of longissimus dorsi in loin	0.556***	31
Ether extract of biceps femoris in bottom round	0.471**	22
Estimated marbling versus		
Ether extract of 9–10–11 ribeye	0.597***	36
Ether extract of longissimus dorsi in loin	0.654***	43
Ether extract of biceps femoris in bottom round	0.668***	45
Ether extract of 9–10–11 ribeye versus		
Ether extract of longissimus dorsi in loin	0.832***	69
Ether extract of biceps femoris in bottom round	0.742***	55

** Significant at the 1% level.
*** Significant at the 0.1% level.

TABLE 3. JUICINESS AND TENDERNESS SCORES AND SHEAR FORCE VALUES IN STEAKS FROM TWO CUTS COOKED BY TWO METHODS

Cut and muscle	Method of cooking	Number of animals	Juiciness scores mean (S.D.)	Tenderness scores mean (S.D.)	Shear force values mean (S.D.)
Loin (L. dorsi)	Broiled	38	6.5 (0.7)	6.5 (1.4)	9.9 (1.8)
	Braised	38	5.2 (0.6)	6.7 (1.5)	9.9 (2.4)
Bottom round (B. femoris)	Broiled	38	7.8 (0.8)	6.9 (1.2)	8.7 (0.9)
	Braised	38	5.5 (0.7)	8.0 (1.1)	5.9 (1.2)

of eye muscle as a measure of fatness and the shear values of rare roasted 9–10–11 rib as the measure of tenderness. The coefficient of correlation between them was $-0.108 \pm .025$ for 728 cattle. In another group of 69 cattle tested by them it was $-0.074 \pm .082$. Barbella *et al.* (1939) using 728 cattle reported that higher juiciness scores of roasted left 9–10–11 rib were associated with the fatter cattle. Fatness was determined by ether extract of edible portion (fat and lean) of the right 9–10–11 rib cut. Wanderstock and Miller (1948) used separable

TABLE 4. SIMPLE CORRELATIONS BETWEEN FATNESS AND THE EATING QUALITY OF THE MEAT

Cut and method of cooking		Independent variables	Dependent variables: Juiciness scores	Tenderness scores	Shear force values
Loin	Broiled	Estimated fat in carcass	0.484**	0.244	−0.249
		Estimated marbling	0.369*	0.266	−0.218
		Ether extract in ribeye	0.512**	0.343*	−0.330*
		Ether extract in this cut	0.367*	0.201	−0.238
	Braised	Estimated fat in carcass	0.104	0.056	−0.221
		Estimated marbling	0.131	0.138	−0.187
		Ether extract in ribeye	0.359*	0.297	−0.344*
		Ether extract in this cut	0.200	0.165	−0.282
Bottom round	Broiled	Estimated fat in carcass	0.220	0.187	−0.146
		Estimated marbling	0.342*	0.348*	−0.279
		Ether extract in ribeye	0.385*	0.501**	−0.351*
		Ether extract in this cut	0.226	0.436**	−0.155
	Braised	Estimated fat in carcass	0.093	0.180	−0.199
		Estimated marbling	0.146	0.370*	−0.326*
		Ether extract in ribeye	0.249	0.545***	−0.524***
		Ether extract in this cut	0.235	0.506**	−0.524***

* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

fat as an index of fatness. In their tests, the lots lowest and highest in fatness averaged 18.3% and 30.5% separable fat, respectively. The tenderness scores for these two lots averaged 5.1 and 5.8, respectively and the juiciness scores 4.5 and 6.1, respectively. Thus the results in the literature indicate that fatness may be somewhat more closely associated with juiciness of rare roasted 9–10–11 ribs than with tenderness of the same cut and also that the association obtained between fatness and tenderness was not as marked as had been supposed earlier.

Broiled loin seems closest to the test method used by Cooperative Meat Investigations (1942). Broiling is a dry heat method of cooking and the test muscle (longissimus dorsi) occurs also in 9–10–11 rib. Using broiled loin as the test method, the association between fatness and juiciness seemed better than between fatness and tenderness, but even the highest of the coefficients was so low that the variation in fatness accounted for less than 30% of the variation in juiciness of broiled loin steaks. Tenderness of broiled loin was associated significantly with only one of the measures of fatness—ether extract in ribeye and it accounted for only about 10% of the variation in tenderness. Since the conclusions reached from work on well-done broiled loin as the test method were in the same direction as those reported in the literature and the association between fatness and tenderness was as good or better, it may be that broiled loin is at least as sensitive for showing the relationship between fatness and tenderness as is the 9–10–11 rib cut roasted rare under the conditions standardized by Cooperative Meat Investigations. The broiled steaks also require much less time to prepare and replications may be obtained with a much smaller weight of meat.

Because estimated fat in the carcass should indicate the separable or gross fat in the carcass and because fatness and tenderness have been thought to be associated for so long (Armsby, 1908, 1917; Bull, 1916; Hall, 1910; Helser, 1929; and Henry and Morrison, 1916), it may be surprising that estimated fat in the carcass was not significantly associated with tenderness in either of these cuts by either method of cooking. Ether extract of ribeye was the only measure of fatness which was significantly associated with tenderness in both cuts and both methods of cooking but the association was greatest in bottom round—usually a cheaper cut of meat than the loin. Even in braised bottom round the variation in ether extract accounted for no more than 30% of the variation in tenderness.

Although none of the coefficients of correlation is high, all are positive and it is probable that fatness as measured by ether extract of ribeye is associated with juiciness and tenderness. Why all measures of fatness or even all measures of marbling were not equally well correlated with juiciness and tenderness is not known. It is especially puzzling why tenderness of loin steaks was not as well correlated with ether extract of loin as it was with ether extract of ribeye. At any rate the agreement between fatness and tenderness is low enough so that it is not surprising that a consumer who buys fat loin steaks or even well marbled loin steaks is sometimes disappointed in their tenderness.

Because none of the coefficients is high, it is doubtful that fatness by itself is responsible for a marked increase in tenderness and juiciness. It is disconcerting that something which has appeared so obvious to so many for so long should be so extraordinarily difficult to prove in the laboratory. This must mean that one or more basic relationships are not being considered. Other factors such as breed, breeding, ration, age, and rate of gain have been suggested as having an influence on the eating quality of meat. Data on some of these factors for these 38 steers are available and will be considered in a subsequent publication.

Summary

Fatness was measured by physical separations, by estimated marbling and by percentage ether extract of trimmed muscles. Juiciness and tenderness scores and shear force values were obtained for each of two cuts cooked by each of two methods from each of 38 animals. Juiciness scores seemed more closely correlated with fatness in broiled loin than in braised loin or in broiled or braised bottom round. Juiciness scores were more closely correlated with ether extract than with the other measures of fatness. Fatness seemed more closely correlated with tenderness scores in bottom round than in loin. Tenderness scores were more closely correlated with ether extract than with the other measures of fatness. All of the correlations were positive and it is probable that ether extract, at least, is associated with juiciness and tenderness of the meat. But none of these coefficients is very high. When loin steaks (usually a higher priced cut) were cooked by broiling, variations in fatness, at best, accounted for about 10% of the variation in tenderness and about 25% of the variation in juiciness. In braised bottom round steaks (usually a cheaper cut than the loin), the variation in fatness accounted for about 30% of the variation in tenderness and about 5% of the variation in juiciness.

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DIGESTION AND ABSORPTION OF VARIOUS CARBOHYDRATES POSTERIOR TO THE RUMINO-RETICULAR AREA OF THE YOUNG BOVINE [1]

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THE possibility of supplementation of the whole milk diet of the young bovine with more economical sources of energy-yielding nutrients has been investigated extensively. Starch is one of the more readily available substances for this purpose. However, it has been observed (Hull and Rettger, 1917; Maynard and Norris, 1922; Norris, 1925; Shoptaw *et al.*, 1937) that calves fed relatively large amounts of this carbohydrate in milk substitutes do not thrive, apparently due to poor utilization of the starch. In previous studies (Larsen, 1952) it was noted that starch (of corn) which was introduced into the abomasum without previous exposure to rumen processes appeared essentially undigested in the feces.

The objective of the present investigation was to determine, indirectly by measuring blood reducing sugar levels and directly by digestibility studies, the ability of the young ruminant to digest starch and related carbohydrates posterior to the rumino-reticular area of the gastrointestinal tract.

Exerimental Procedure

Three male calves (two Milking Shorthorns and one Holstein), ranging initially from 8 to 9 months of age, were provided with both rumen and cecal fistulas. The rumen fistula was located in the center of the left paralumbar fossa and the cecal fistula was located in a similar position on the right side.

Prior to beginning the investigation the animals were allowed water and green leafy alfalfa hay free choice and were fed approximately 2.5 lb. of a concentrate mixture [5] daily per calf. Three days before

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[5] Corn, 400 parts; oats, 500; wheat bran, 200; soybean oil meal, 100; linseed oil meal, 200; steamed bone meal, 28; and iodized salt, 14 parts.

initiation of experimental feeding the animals were placed on a whole milk diet and solids were evacuated from the rumens. Five to 5.5 lb. of experimental diets (table 1) were fed daily. This was calculated to be sufficient for maintenance plus 1 lb. gain in weight daily.

As shown in table 1, diets 2, 3 and 4 were basically the same as diet 1 except the corn was replaced with powdered corn starch, maltose in the form of dried malt syrup, and dextrose, respectively, plus soybean oil meal. In all rations the crude protein content was approxi-

TABLE 1. COMPOSITION OF THE EXPERIMENTAL DIETS

Ingredient[a]	Diets			
	1	2	3	4
	lb.			
Ground yellow corn	30.8			
Soybean oil meal	5.2	10.2	8.5	10.2
Alfalfa meal	1.0	1.0	1.0	1.0
Meat and bone scraps	2.0	2.0	2.0	2.0
Starch		21.6		
Dried malt syrup			28.1	
Dextrose				21.6
Total	39.0	34.8	39.6	34.8

[a] Minerals and vitamins added to each diet included: $MnSO_4$, 2.55 gm.; $FeSO_4$, 6.50 gm.; $CuSO_4$, 0.20 gm.; $ZnSO_4$, 0.21 gm.; K_2HPO_4, 0.77 gm.; KI, 0.04 gm. and $Co(C_2H_3O_2)_2$, 0.08 gm.; vitamin A, 150,000 I.U.; vitamin D, 15,000 I.U.; choline, 16.88 gm.; riboflavin, 0.057 gm.; niacin, 0.75 gm.; and pantothenic acid, 0.30 gm.

mately 16%. The dried malt syrup [6] was composed of maltose, 76 to 78%; dextrins, 11 to 15%; protein, 4 to 5%; and ash, 1.5%. The starch was a commercial grade of corn starch.[7]

Mineral salts and vitamins included in the diets were premixed with soybean oil meal in a Hobart mixer. For digestibility measurements chromium oxide was added to the diets at approximately the 1% level. All constituents not in a powdered state were ground (prior to mixing) to pass a 0.04 inch screen.

Trial I

Each animal was fed an experimental diet for 2-day intervals since longer periods on these diets resulted in a syndrome characterized by weakness, loss of weight and occasional diarrhea (Larsen, 1952). Between the 2-day feeding periods, whole milk (15 lb. daily) was fed for

[6] Produced by Flieschman Distilling Corporation, New York, N. Y.
[7] Produced by A. E. Staley Manufacturing Company, Decatur, Illinois.

2-day intervals by nipple bucket. No milk spillage from the esophageal groove into the rumen was noted. The feeding regime for each animal is shown in table 2.

Experimental diets were fed twice daily, approximately 2.5 lb. of dry mixture per feeding, in the form of a slurry (1 part dry mixture: 1 part warm water) mixed thoroughly and pumped from a bucket into the omaso-abomosal cavity through a hose placed in the reticulo-omasal orifice. No regurgitation of the experimental diet into the rumen was noticed. In previous studies (Larsen, 1952) it was found that with this type of feeding 8 to 10 hours were required for feed to traverse the gastrointestinal tract posterior to the rumen. Therefore, to minimize carry-over effects (from previous treatments or from the milk diets)

TABLE 2. DIETARY REGIMES FOR ANIMALS IN TRIAL I

Period	Calf number		
	1	2	3
	(Diet No.)		
First	3	2	1
Second	4	3	2
Third	1	4	3
Fourth	2	1	4

cecal and fecal samples were collected only on the second day of each experimental feeding period. The rumen was essentially empty during the course of the experiment except for water which was allowed free choice.

During the second day of each feeding period blood samples were collected from the jugular vein at 0, 1, 2, 4, 6, 8 and 10 hours after the morning feeding. Sodium fluoride and potassium oxalate were employed to prevent glycolysis and coagulation. The blood samples were analyzed for reducing sugars later in the same day. Cecal and fecal samples were collected at 8 and 10 hours after the morning feeding of the second day. The two cecal samples were stored in separate containers while the fecal samples were composited. Ingesta samples were autoclaved (15 p.s.i.g. for 10 minutes) immediately after collection to inactivate enzymes present and subsequently were frozen and held in this condition until analyzed.

Blood reducing sugar was measured by the method of Somogyi (1945). The chromium oxide in the feeds, cecal ingesta and feces was determined by the method of Schürch *et al.* (1950). A standard

(calibration) curve was prepared with known quantities of chromium oxide.

Carbohydrate in the feeds and ingesta was determined by an adaption of the procedure of Dimler *et al.* (1952). Prior to the actual carbohydrate determination, the samples were treated as follows: The frozen sample was thawed rapidly in a warm water bath, a 5-gm. aliquot was mixed thoroughly with water in a Waring blendor for 1 minute, and the resulting mixture was filtered through a Büchner funnel and made up to 250 ml. with water. A 25-ml. aliquot was preserved with phenylmercuric acetate (1 p.p.m.) and was refrigerated until analyzed. The original procedure was followed from this point except that the carbohydrate solution was added to the chromatogram starting line in 5 microliter aliquots instead of one. Recoveries with this procedure were consistently in the range of 97 to 100%.

Starch determinations were made by combining acid hydrolysis (A.O.A.C., 1950) with the anthrone sugar determination (Dimler *et al.*, 1952). Five grams of material to be analyzed were subjected to acid hydrolysis; the resulting solution was filtered hot and was made up to 250 ml. with water. A 25-microliter aliquot of the filtered solution was diluted to 10 ml., resulting in a carbohydrate content ranging from 3 to 30 mcg. per ml. Three ml. of this solution were used in the carbohydrate determination by the anthrone method.

Trial II

In this phase of the work, animals 1 and 2 of the previous trial were used to ascertain rates of absorption of glucose from various areas of the gastrointestinal tract. Subsequent to trial I the animals were allowed to revert to normal functioning ruminants with hay and concentrates fed free choice. Before starting trial II the rumen of each animal was washed out with warm water. Then the animals were anesthetized (15 ml. of a 6% Nembutol solution per 100 lb. body wt.) and subsequently a midline incision was made from the point of the sternum to a point about 18 inches posterior. In calf No. 1 the ventral lobe of the rumen, the anterior portion of the ilium and the posterior portion of the cecal diverticulum were ligated. Into the ventral rumen lobe and into each of the isolated portions of the intestinal tract were injected 200 ml. and 50 ml., respectively, of a 50% glucose solution. Samples of venous blood from each of the areas were taken immediately before the sugar injection and at 20-minute intervals thereafter for a period of 80 minutes.

In most respects animal No. 2 was treated similarly to No. 1. In addition, however, it seemed desirable to determine the effect of Nembutol upon the blood reducing sugar levels. Therefore, a blood sample was taken from the jugular vein prior to injection of the anesthetic and at 20-minute intervals throughout this phase of trial II. The ventral rumen lobe and sections of the ileum, cecum and jejunum, respectively, were ligated and a glucose solution was injected into each of the isolated portions. Venous blood samples for subsequent blood reducing sugar analyses were taken from each of the areas at 20-minute

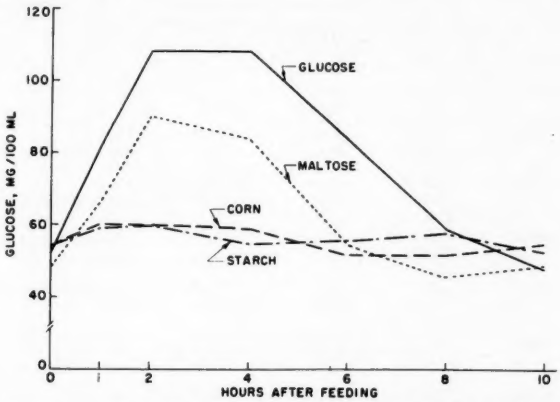

Figure 1. Changes in blood reducing sugar levels following introduction of various carbohydrates into the omaso-abomasal area.

intervals for an 80-minute period. Since the cecum was inaccessible during this period, injection into this organ was made at the end of the initial 80-minute period and cecal absorption was observed for a 40-minute period thereafter.

Results

Trial I

Figure 1 indicates the blood reducing sugars of the intact animals subsequent to ingestion of the four diets. These curves, which represent the mean values of all animals, demonstrate that the feeding of corn and starch diets effected no appreciable rise in blood reducing sugar. In contrast, following glucose and maltose feeding the blood reducing sugar levels rose rapidly during the first 2 hours and declined sharply after the fourth hour with maximum values being attained between the

second and fourth hours. The mean blood reducing sugar levels following maltose feeding did not increase as much as and returned to the initial level earlier than those following glucose feeding.

The results of the digestibility studies with corn and starch are shown in table 3. Because of an apparent collapsed cecum no samples

TABLE 3. COMPOSITION OF FEED, CECAL AND FECAL SAMPLES FOR CORN AND STARCH DIETS [a]

	Sample	Dry matter	Carbohydrate	Apparent digestibility of carbohydrate	Fecal characteristics
		%	% of D.M.	%	
Feed	Corn	90.1	52.2		
	Starch	89.9	56.6		
Calf 1	Cecal C [b]	15.6	64.4	5.3	Fluid
	S [c]	22.8	81.0	−37.7	Alternately fluid and pasty
	Fecal C	15.7	78.8	− 8.1	Fluid
	S	15.6	77.6	16.0	Alternately fluid and pasty
Calf 2	Cecal C	28.6	77.7	− 3.9	Alternately fluid and pasty
	S	24.5	88.7	− 8.6	Fluid
	Fecal C	17.9	64.7	−12.3	Alternately fluid and pasty
	S	21.8	61.8	59.7	Fluid
Calf 3	Cecal C	....	....	....	.....
	S	16.1	96.0	−48.1	Very fluid
	Fecal C	20.1	68.1	24.3	Very fluid
	S	18.6	87.7	−10.2	Very fluid

[a] No appreciable reducing sugar was found in feces and cecal contents following glucose and maltose feeding.
[b] Corn diet.
[c] Starch diet.

of cecal origin could be collected from animal No. 3 during corn feeding. No values are presented here for the digestibility of the glucose and maltose diets, since, when these diets were fed, no appreciable reducing sugars appeared in the cecal ingesta or in the feces. These data and those presented in figure 1 indicate that glucose and maltose were readily absorbed, the latter presumably after hydrolysis. Profuse scouring was observed in all cases during the feeding of the glucose and maltose diets.

It can be noted in table 3 that the percent carbohydrate in the dry matter is consistently higher in the cecal ingesta and fecal excreta than in the diets. Also noticeable is the great variation in carbohydrate digestibility, an observation which is difficult to explain. However,

diarrhea of varying intensity usually occurred on both diets and this may have been responsible, at least in part, for the variations noted.

Carbohydrate digestibility figures are presented in more detail in table 4. Also presented are the "net changes" in apparent digestibility between the cecum and the point of excretion. The "net change" for the corn diet was consistently negative while that for starch was consistently positive. At the cecal level digestibilities of the starch diet were highly negative. By using the mean of the 8 and 10 hour cecal values to estimate "net change" these approximations for corn and starch are

TABLE 4. DIGESTIBILITY OF CORN AND STARCH DIETS AS INDICATED BY CECAL AND FECAL SAMPLES

		Corn		Net	Starch		Net
	Sample	Cecal	Fecal	change[a]	Cecal	Fecal	change[a]
Calf	8 hr.	11.80	− 8.06	−19.86	−43.30	16.03	59.33
1	10 hr.	− 1.20		− 6.86	−32.03		48.06
	Mean	5.30	− 8.06	−13.36	−37.67	16.03	53.70
Calf	8 hr.	−11.18	−12.30	− 1.12	2.88	59.66	56.78
2	10 hr.	3.33		−15.63	−20.08		79.74
	Mean	− 3.93	−12.30	− 8.37	− 8.60	59.66	68.26
Calf	8 hr.		24.25		−59.10	−10.17	48.93
3	10 hr.				−37.10		26.93
	Mean		24.25		−48.10	−10.17	37.93
All calves	Mean	0.68	−10.18[b] 1.30[c]	−10.86	−31.46	21.84	53.30

[a] Net change between mean of the cecal values and the fecal values.
[b] Excluding calf 3.
[c] Including calf 3.

negative and positive, respectively. The mean net changes for the corn and starch diets were approximately −11 and 53%, respectively. The small difference between the over-all means of cecal and fecal values on the corn diet and the wide difference for the starch diet indicates small and large changes, respectively, in apparent digestion in the large intestine.

Trial II

Figure 2 shows the changes in blood reducing sugar levels following injection of glucose into ligated sections of exteriorized viscera of the bovine. Blood samples were obtained from efferent blood vessels of the respective visceral sections.

Following injection of glucose into the jejunum and ileum a relatively constant rise was noted in each section; however, the rate of increase was considerably less in the latter. Cecum and rumen values revealed a definite rise during the initial 20-minute interval. Blood

reducing sugar levels in jugular blood were relatively constant during the period of observation.

Discussion

In the present investigation the marked differences in carbohydrate digestibility between the mono- and disaccharide diets on the one hand and the corn and starch diets on the other possibly may be attributed to the lack of intestinal or pancreatic amylases or to the specificity of amylases which might be present. The large amounts of starch

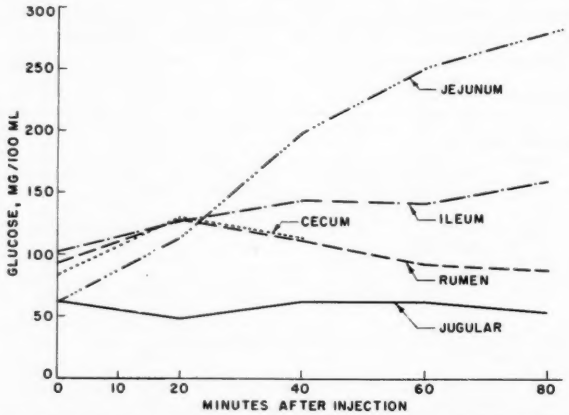

Figure 2. Changes in blood reducing sugar levels following injection of glucose solution into ligated sections of viscera.

in the feces and cecal material of calves which were fed corn or starch diets support this hypothesis. The calculated digestibilities of the carbohydrate, though variable, indicate extremely limited starch utilization. Certain investigators (Baker, 1939, 1942; Van Der Wath, 1948) suggest that a large portion of the carbohydrate ultimately utilized by the ruminant is made available via a bacterial polysaccharide, and other work (Elsden and Phillipson, 1948; Phillipson and McAnally, 1942; Schambye, 1951) indicates that very little carbohydrate in its ingested form reaches the lower gastrointestinal tract. If the former were the case, amylase specificity could be possible; and if the latter were true, total absence of amyolytic enzymes might be possible. It has been shown, however, that substances possessing

amylolytic activity are produced by the intestinal glands as well as by extracts of the submucosa of certain other sections of the intestinal tract (Foster, 1867; Bergmann *et al.*, 1924). This activity was not measured quantitatively by these investigators. The relative strength of pancreatic amylase of various species was attributed to the degree of mastication and to the extent of decomposition of carbohydrate by symbiotic bacteria. Apparently, considerable uncertainty exists as to the activity and actual quantitative production of amylase in the intestine.

The net digestibility changes between the cecum and the rectum (table 4) for all animals on both diets were consistently positive for starch and negative for corn. These values would seem to imply an increase in digestibility for the former and a decrease for the latter. The contrasting situation exhibited in this case cannot be explained satisfactorily on the basis of microfloral growth in the large intestine, although this may be a major factor, but it is of interest to note that the situation was consistent within diets. The apparent digestion of starch in the large intestine may complicate interpretation of digestion trials (involving starch) in young ruminants.

Variable chromium oxide percentages in the dry matter possibly may be attributable to the fluidity of the feces. This chromium oxide variation in turn may be partly responsible for the negative digestibility values.

Indirect measurement of starch digestion (by blood reducing sugar levels) posterior to the rumino-reticular cavity revealed very little starch breakdown in the corn and starch diets. This indicates that the ruminant is handicapped in its ability to derive energy from the starch fraction of the natural grain without the assistance of a normally functioning rumen. The high incidence of diarrhea during omaso-abomasal feeding suggests that factors (mechanical or chemical) inherent in the natural feed may possibly provoke diarrhea.

Portions of the tract showing marked absorption of glucose were the jejunum and ileum, commonly believed to have the greatest absorptive capacity (figure 2). However, it is recognized that the absorptive capacity of an animal under the stress imposed in trial II might be altered and thus the results may not be entirely indicative of normal responses. The mucosal lining of the tract could have been altered by the high concentration of glucose (50%) injected into the tract, thereby encouraging the absorption process. Absorption from the intestinal lumen apparently takes place by a phosphorylation-dephosphorylation reaction

(Hele, 1950), but under conditions of this experiment the process of osmosis or filtration conceivably could have contributed to the sharp rise in the blood reducing sugar level of the jejunum. However, those who have used concentrations in the ranges employed herein do not attribute absorption to these forces but rather to a controlled absorptive mechanism (Hagen, 1952; Magee and Reid, 1931; Ravdin *et al.*, 1933). A factor contributing to the continued high concentrations of blood reducing sugar levels may have been blood stasis in the organ, but this seems unlikely since increases in other isolated segments, in the present study, were not nearly so great.

Blood reducing sugar values for the cecum and rumen veins revealed a maximum increase 20 minutes after injection with the levels thereafter slowly receding toward the initial values. Although these results suggest that glucose may be absorbed from both the rumen and cecum, the limited data and the stress condition suggest the need for caution in interpretation. A number of investigators (Elsden and Phillipson, 1948; Huffman, 1953; Phillipson and McAnally, 1952; Schambye, 1951) are of the opinion that the ruminant is rarely, if ever, called upon to obtain energy in large amounts from glucose but rather derives an adequate supply from the lower volatile fatty acids. Apparently glucose, the primary blood reducing sugar, is obtained principally from the propionic acid in the rumen (Elsden and Phillipson, 1948; Reid, 1950; Ringer, 1912). The results of the present study indicate that the ruminant is capable of glucose absorption even though this species does not normally have available for absorption large quantities of glucose from dietary carbohydrate.

The results of this experiment suggest the need for isolation and characterization of the amylolytic enzymes in the gastrointestinal tract of the bovine. This would make possible subsequent comparison to similar enzymes in other domestic animals. Further, this work suggests the need for examination of the accuracy of the chromium oxide indicator technique in studies of this nature where consistency of ingesta is abnormal.

Summary

Three young ruminating dairy calves with ruminal and cecal fistulas were fed four experimental diets directly into the omaso-abomasal cavity to determine the ability to digest and absorb carbohydrates from four sources: glucose, maltose, starch and corn. Digestibility studies

and blood reducing sugar levels indicated that glucose was readily absorbed and maltose was readily (hydrolyzed and) absorbed. Similar criteria demonstrated very little digestion of starch and of carbohydrate of corn.

In absorption studies with isolated visceral sections it was noted that glucose was absorbed readily from the jejunum and ileum while the rumen and cecum were less efficient; however, all sections absorbed glucose.

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EFFECT OF VALERIC AND ISOVALERIC ACIDS ON STRAW UTILIZATION BY STEERS

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BRANCHED and straight-chain fatty acids have recently been shown (Bryant and Doetsch, 1955) to be required by the rumen cellulose-digesting bacterium, *Bacteroides succinogenes,* and some of the fatty acids stimulate the *in vitro* fermentation by mixed suspensions of rumen bacteria (Bentley *et al.,* 1954). These discoveries suggest that small amounts of appropriate acids might improve utilization of poor quality roughages. This was tested in a straw-feeding experiment using steers which had previously received a maintenance ration.

Experimental Procedure

During a period of 183 days eight steers received rations of the following percentage composition (Erwin *et al.,* 1955): wheat straw 82, inedible animal fat 7, barley 4.5, milo 1.5, oats 0.73, beet pulp 1.47, salt 1, and urea 1.8. At the end of this time the steers were divided into a control and experimental group and were continued on the above basal ration for an additional 38 days during which the experimental animals received in addition 8.44 gm. of valeric and 2.75 gm. of isovaleric acid per 60 lb. of feed.

The weight of feed consumed, the initial and final weights of the steers, and the crude fiber digestibility were determined. In addition, the rate of fermenation by 10 gm. of rumen contents from each animal was measured.

Results

The weight determinations and digestibility results are shown in table 1. There was no significant difference in the weight changes of experimental and control animals. Both groups lost weight at about the same rate as during the preceding feeding trial.

In order to determine whether the rumen fermentation had been influenced by the acid additions a sample of rumen contents was removed from each animal and the fermentation rate tested, using the manometric

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TABLE 1. EFFECT OF VALERIC AND ISOVALERIC ACIDS ON WEIGHT GAINS AND CRUDE FIBER DIGESTIBILITY IN STEERS

Animal No.	Ration	Weight June 2	Weight July 10	Gain	Feed consumed	Crude fiber digestibility
		lb.	lb.	lb.	lb.	%
29	basal	615	594	−21		41
32	basal	580	580	0	720	41
33	basal	575	574	−1	average	35
35	basal	675	674	−1		58
25	basal + acids	795	808	13		41
27	basal + acids	620	616	−4	730	38
30	basal + acids	685	662	−23	average	34
34	basal + acids	700	694	−6		61

method previously employed (Hungate *et al.*, 1955, 1955a). The pertinent data are shown in tables 2 and 3.

When no substrate was added, the fermentative activity as measured by the pressure increase due to gas evolution was quite low, as seen in columns 5 and 6 of table 2 and columns 3 and 4 of table 3. The average rate for rumen contents from animals which received no acids in the feed was greater, 20.6 mm. per hr., S.E. 2.9, than in the experimental group, 11.7 mm. per hr., S.E. 1.8. This difference is significant at the 5% level and suggests that the rumen fermentation rate in the steers fed acid was actually less per unit volume than in the controls. In contrast to the above low fermentation rates, rumen contents from a steer on a 50% straw-32% grain ration showed manometric pressure increases of about 80 mm. per hr.

The relative amounts of methane (residual gas, last column of table

TABLE 2. RELATIVE FERMENTATION RATES OF RUMEN CONTENTS FROM STEERS ON 82% STRAW WITH AND WITHOUT ADDED VALERIC AND ISOVALERIC ACID [a]

Animal No.	Addition to feed	Total press. inc. during incubation		Pressure increase during first hour		Residual gas after absorption with NaOH	
		A	B	A	B	A	B
		mm.	mm.	mm.	mm.	ml.	ml.
30	acids	124	...	17	..	3.4	...
34	acids	116	121	16	16	3.5	3.6
35	none	188	187	29	28	5.4	5.4
33	none	152	150	24	24	4.5	4.6

[a] Conditions of the experiment: Ten gm. rumen contents plus 30 ml. balanced salt solution, including 0.5% $NaHCO_3$, (Hungate, 1950) in each vessel. Carbon dioxide as gas phase. Vessel volumes about 160 ml. Mercury in the manometers. The CO_2 was absorbed in alkali at end of the experiment and the residual gas (methane) measured and corrected to standard temperature and pressure. Duration of experiment 448 minutes.

2) are about those expected from the rates of fermentation as measured by pressure increase during the experiment. More methane was formed by rumen contents from the controls. Microscopic examination of the rumen contents disclosed a smaller concentration of protozoa in the animals fed the acids.

In view of the similar weight changes of the two groups it seems doubtful whether the actual total fermentation differed. The feed was consumed more rapidly by the acid-fed animals and may already have undergone its maximum fermentation before samples were collected. It is also possible that the rumen volumes in the two groups differed. At least, the experimental results do not support the view that addition of valeric and isovaleric acids stimulated the rumen fermentation when straw was the chief constituent of the ration.

TABLE 3. EFFECT OF ACID-FEEDING AND SUBSTRATE-ADDITION ON RELATIVE RATES OF FERMENTATION OF INCUBATED RUMEN CONTENTS [a]

Animal No.	Addition to feed	Pressure increase during 35 minutes before addition of hay		Pressure increase during 165 minutes after addition of hay		Residual gas after absorption with NaOH	
		A	B	A	B	A	B
		mm.	mm.	mm.	mm.	ml.	ml.
25	acids	4	4	229	233	3.9	...
27	acids	6	5	220	208	4.1	4.0
29	none	6	8	206	180	4.1	4.1
32	none	10	10	241	227	4.8	4.8

[a] Conditions of the experiment were as in table 2 except that after 35 minutes incubation 1 gm. dried powdered grass-alfalfa hay was added to each vessel from the sidearms.

The figures in columns 5 and 6 of table 3 show that when fresh substrate in the form of hay was added to the incubated rumen contents the rate of fermentation was about the same in all animals. This is interpreted as showing that the experimental and control animals contained about the same concentration of total microbial protoplasm.

Discussion

The negative results of the study may be due to lack of fermentable carbohydrate in the ration. With little carbohydrate the rate of fermentation without added acids may be adequate to break down all digestible substrate. Decomposition of the protein in the ration grains may have supplied sufficient branched and straight-chain acids to permit the satisfactory fermentation of the small amount of available carbohydrate. The crude fiber digestibility (last column of table 1) is lower

than in good quality roughage and may represent the level at which only maintenance is possible.

Since it was desired to keep feed consumption in the two groups identical, a weighed quantity was fed each day and the amount consumed regulated according to animal weight. The feed containing the acids was consumed much sooner each day than was the ration of the controls. With *ad lib* feeding, the animals on the ration with acid would probably have consumed more feed. The effect on the appetite suggests that the acids may play some role in nutrition and makes desirable further experiments with a more suitable source of carbohydrate.

Summary

The weight gains and rumen microbial activity were not significantly different in steers fed a high wheat straw ration with and without small amounts of valeric and isovaleric acids. This negative result may have been due to lack of digestible carbohydrate in the ration. The appetite was stimulated in the animals receiving the acids.

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A LABORATORY TECHNIQUE FOR MEASURING PHOSPHORUS AVAILABILITY OF FEED SUPPLEMENTS FED TO RUMINANTS [1, 2]

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IT is well recognized that ruminants require dietary phosphorus for growth and maintenance. Availability of phosphorus from various dietary sources differs considerably in poultry (Gillis *et* al., 1954; Wilcox *et al.*, 1954) and in swine (Combs, 1955). Preliminary studies with cattle (Long *et al.*, 1955) and with lambs (Ammerman *et al.*, 1955) also indicate difference in phosphorus availability with a limited number of feeding supplements tested. One of the problems of investigating phosphorus availability of the large number of supplements fed to cattle and sheep is the expense of carrying out long time feeding and metabolism experiments with these animals. Since rumen microorganisms require phosphorus (Burroughs *et al.*, 1951) for efficient cellulose digestion, it seemed plausible that an artificial rumen technique could be developed for measuring phosphorus availability which might serve as an index of phosphorus availability of supplements fed to ruminants. It is the purpose of this paper to describe an artificial rumen technique adapted for measuring phosphorus availability of supplements commonly used in ruminant rations.

Materials and Methods

The technique employed consisted of measuring cellulose digestion in a series of small fermentation tubes to which graded amounts of phosphorus were added at the beginning of a 24-hour fermentation period. In order to obtain the greatest response from phosphorus upon cellulose digestion it was necessary to first carry out a 24-hour preliminary fermentation making use of a washed suspension of rumen microorganisms in a phosphorus-deficient medium. This preliminary fermentation was carried out in a large flask in which special precautions were taken to supply all nutrients except phosphorus required by rumen microorganisms for efficient cellulose digestion. In addition to supplying the known nutrient requirements of energy, nitrogen and minerals, the

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technique was greatly facilitated by supplying unknown nutrients in the media such as casein or feather meal specially hydrolyzed (Hall *et al.*, 1954).

The preparation of a washed suspension of rumen microorganisms was similar to that described by Cheng *et al.* (1955). Two liters of rumen fluid were first obtained from a fistulated steer by straining rumen ingesta through four layers of number 50 cheese cloth into previously warmed thermos bottles. The strained rumen fluid was next centrifuged in a Servall angle centrifuge at a speed of about 1000 r.p.m. for two minutes. This process sedimented partially digested feed particles and protozoa which were not completely removed by the cheese cloth. The supernatant was next centrifuged in a Sharples centrifuge at a speed of about 25,000 r.p.m. The bacteria in the bowl of the centrifuge with the exception of about one-fourth inch in the bottom were collected and suspended in 1 L. of distilled water saturated with carbon dioxide gas.

This bacterial suspension was again put through the Sharples centrifuge to sediment the bacteria and remove food nutrients that might be clinging to the surface of bacterial cells. The resulting sediment was added to 1 L. of phosphorus deficient medium as described in table 1 along with 5 gm. of finely divided cellulose and 15 ml. of a special 5% hydrolyzate of feather meal or vitamin-free casein.

The preliminary 24-hour fermentation of this suspension was carried out in a 2 L. Erlenmeyer flask suspended in a water bath maintained at 39° C. This phosphorus-depletion fermentation was carried out under anaerobic conditions maintained by passing a constant stream of carbon dioxide gas through the suspension.

At the end of the 24-hour period, half of the contents of the flask was discarded and the flask made up to 1 L. by adding phosphorus deficient medium and 5 gm. of cellulose. Aliquots of 20 ml. of the suspension were pipetted into 75 ml. centrifuge tubes which also served as fermentation tubes. To each tube was added 0.3 ml. of hydrolysate of feather meal or casein. In developing a standard curve, graded amounts of a standard phosphorus solution were added to the tubes. The standard solution consisted of 2 parts dibasic sodium phosphate and one part monobasic potassium phosphate. Graded levels of phosphorus feeding supplements were added to other series of tubes in determining phosphorus availability. These tubes were each fitted with a stopper with inlet and outlet glass tubings for bubbling a slow constant flow of carbon dioxide gas for purposes of agitation and maintaining anaerobic conditions. The tubes

were fermented at 39° C. in a water bath. At the end of the 24-hour fermentation, cellulose digestion was determined on the entire tube contents as previously described by Cheng *et al.*, (1955).

Five phosphorus supplements were assayed in checking this method of determining phosphorus availability. These supplements were composite dicalcium phosphate, acidulated product of phosphate, steamed bone meal, Curacao rock phosphate and colloidal clay. All of these products are presently being used in ruminant rations and were supplied by the National Mineral Feeds Association. Samples of these supplements were ground and suspended in water. They were made up into dilutions containing several levels of inorganic phosphorus. One ml. of suspension was added per tube and all determinations were made in duplicate.

TABLE 1. COMPOSITION OF PHOSPHORUS DEFICIENT BASAL MEDIUM

Constituent	Amount (gm. per 2 L.)
Sodium bicarbonate	3.5
Potassium chloride	0.75
Sodium chloride	0.75
Magnesium sulfate	0.15
Copper sulfate (5 H_2O)	0.002
Manganese sulfate	0.0004
Zinc sulfate	0.00008
Ferrous sulfate (7 H_2O)	0.075
Cobalt chloride (6 H_2O)	0.002
Urea	2.000

The vitamin-free casein and feather meal were partially hydrolyzed with hydrochloric acid. A 5% solution was prepared by adding 25 gm. of either feather meal or vitamin-free casein to 500 ml. of 3 N hydrochloric acid. The mixture was autoclaved at 15 lb. pressure for 4 hours. The pH of the acid hydrolyzate was adjusted to between 6.5 and 6.8 by adding sodium hydroxide. The insoluble material was removed by centrifugation and filtration.

Three fistulated steers were used in obtaining samples. The rations fed differed somewhat from time to time. Each was fed twice daily, once at 9 A.M. and once at 5 P.M. Rumen samples were collected prior to the morning feed.

Results and Discussion

The method employed produced an approximate linear relationship between the amount of phosphorus added (up to 40 mcg. per ml.) to the

deficient medium and the amount of cellulose digested by rumen microorganisms. This relationship could be regularly produced with inoculum obtained from different steers and from steers fed different rations. Figure 1 gives typical results of three fermentations carried out on different days using the standard phosphorus solution.

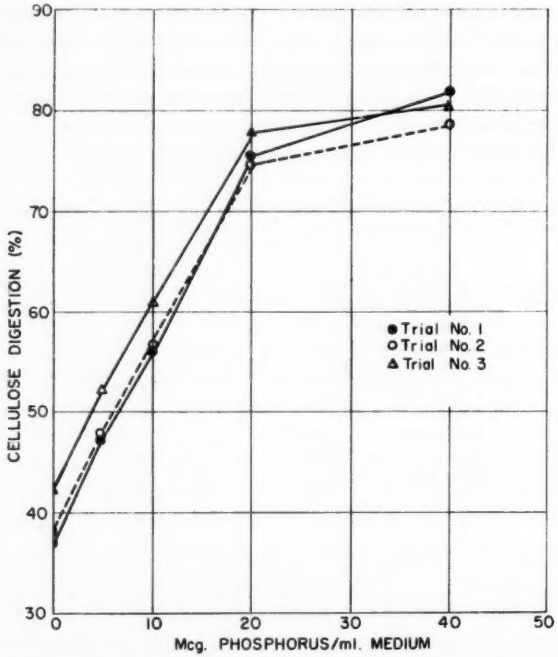

Figure 1. Response of different amounts of standard phosphorus solution upon in vitro cellulose digestion by rumen microorganisms.

It will be noted that the slope of the curves obtained varied somewhat from one determination to another. This is to be expected since the starting inoculum varies somewhat as to numbers and kinds of organisms present even though approximate standardized conditions were employed in sampling rumen ingesta. Since this situation exists it is necessary in testing phosphorus availability of a feeding supplement to run a standard response curve at the same time the supplement is being tested. Figure 2 presents a series of curves with different phos-

phorus feeding supplements and the standard phosphorus solution all run on the same day under identical conditions.

The levels of phosphorus used were calculated in terms of micrograms of phosphorus per ml. of medium. It is seen that cellulose digestion increased from 34% in the basal medium to 80% when 80 mcg. per ml. of standard phosphorus was added to the medium. Among the supplements assayed, composite dicalcium phosphorus appeared to be almost as available a source of phosphorus as the standard phosphate solution while the acidulated product was somewhat less available

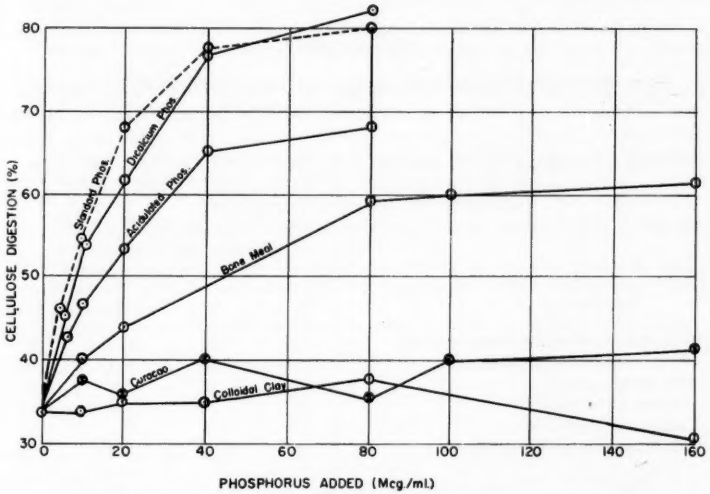

Figure 2. Assay of phosphorus availability to rumen microorganisms.

followed by steam bone meal. Curacao rock phosphate as well as colloidal clay, on the other hand, appeared to be less available. The approximate rating of phosphorus availability of these samples using this method of assay is illustrated in table 2.

It should be pointed out that this rating is tentative and is presented only for purposes of illustrating the method of determining phosphorus availability. It does not necessarily represent the absolute availability to ruminants. It is conceivable that different sources of a given supplement will vary in availability either due to method of manufacture or other considerations little understood at present. The possibility exists that rumen microorganisms in livestock feeding prac-

tice undergo adaptation to different types of phosphorus supplements which allow them to make efficient use of a phosphorus supplement following the adaptation. These considerations require further study; however, the preliminary results obtained by Long *et al.* (1955) with dicalcium phosphate and colloidal clay indicate rather close agreement between the present laboratory method of determining phosphorus availability and availability studies with cattle involving growth, blood and bone analysis. Further experimental work is in progress in attempts to correlate phosphorus availability in cattle and sheep with this laboratory method.

Summary

An *in vitro* fermentation with rumen microorganisms (artificial rumen) was developed to measure phosphorus availability of cattle and sheep feed supplements. Cellulose digestion in a series of fermentation tubes was related to graded amounts of phosphorus added at the beginning of a 24-hour fermentation period. A preliminary fermentation making use of a washed suspension of rumen microorganisms in a phosphorus-deficient medium greatly facilitated the assay fermentation. Also the use of specially hydrolyzed casein or feather meal added to the medium proved helpful in obtaining the greatest response of phosphorus additions upon cellulose digestion. Among the supplements assayed it was found that composite dicalcium phosphate appeared to be as available as a standard sodium-potassium phosphate mixture. An acidulated phosphorus product and steamed bone meal were intermediate in phosphorus availability while Curacao rock phosphate and colloidal clay appeared to be less available. The merits of this laboratory technique

TABLE 2. APPROXIMATE RATING OF THE AVAILABILITY OF PHOSPHORUS FROM DIFFERENT SUPPLEMENTS TESTED BY ARTIFICIAL RUMEN TECHNIQUE

Supplements	Levels of added phosphorus mcg. per ml. of medium			
	10	20	40	Av.
Sodium and potassium phosphate, std.	100	100	100	100
Composite dicalcium phosphate	98	82	100	93
Acidulated product	61	56	74	64
Steamed bone meal	28	29	29	28
Curacao rock phosphate	17	5	13	12
Soft phosphate with colloidal clay	0	2	3	2

in measuring phosphorus availability were discussed with respect to their transposition to feeding practice in cattle and sheep.

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THE ROLE OF CARBOHYDRATES IN UREA UTILIZATION, CELLULOSE DIGESTION AND FATTY ACID FORMATION

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UREA utilization as well as cellulose digestion in the rumen are dependent on the nutritive status and requirements of the rumen microorganisms. The findings of Burroughs *et al.* (1950) suggest that the maximum utilization occurs only when energy and minerals are adequate in supply. Mills *et al.* (1942) showed that the addition of starch to a ration of urea and hay resulted in increased protein synthesis in the rumen of a heifer. Mills *et al.* (1944) further demonstrated that starch was superior to molasses *in vivo* in promoting protein synthesis from a ration containing urea. Studies *in vitro* by Pearson and Smith (1943) also pointed to the need for an ample supply of available carbohydrate, such as starch and sugars, for the conversion of urea nitrogen to protein nitrogen. In an extensive study with various carbohydrate materials, Arias *et al.* (1951) demonstrated the need for some readily available carbohydrate to promote efficient cellulose digestion and urea utilization.

Besides helping the fixation of urea nitrogen in the rumen, the carbohydrates yield fatty acids, which are vital to the ruminant as energy, intermediates for tissue biosynthesis and milk production. The composition of feed ingredients have direct bearing on the amount and distribution of volatile fatty acids formed in the rumen (El-Shazly, 1952; Gray *et al.*, 1951). The findings of Elsden (1945), Marston (1948), and Gray and Pilgrim (1951, 1952) demonstrate the high level of propionic acid formed by cellulose fermentation in the rumen. Belasco (1954b, c) showed a relationship between high cellulolytic activity and propionic acid production.

The objective of this study was to explore further the role of various carbohydrates on urea utilization and the differences in production and distribution of the fatty acids resulting from the fermentation of these energy sources.

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Experimental

The artificial rumen technique used was essentially the same as that described by Burroughs *et al.* (1950) and Belasco (1954a). Each fermentation flask (1 liter) was charged with 0.6 gm. of cane molasses ash, test amounts of cellulose (Solka Floc SW 40A), corn starch, xylan, pectin, or dextrose, 0.8 gm. urea, a nutrient salt solution approximating the composition of sheep saliva (McDougall, 1948), 450 ml. rumen liquid inoculum and distilled water to make up the 900 ml. volume. The systems were gassed with carbon dioxide and maintained at 39° C. in a constant temperature bath. A fermentation pH of 6.2 to 6.9 was maintained with 4N aqueous sodium carbonate. At the end of each 24-hour period, representative samples were withdrawn for analysis of ammonia and urea by A.O.A.C. methods, and cellulose by the method of Crampton and Maynard (1938). At this time also, the volume of the flask contents were adjusted to 900 ml. with distilled water, of which 450 ml. was used as inoculum for another flask containing new nutrients, in amounts identical with those used for the first fermentation period. This subculturing and analysis was repeated for four successive 24-hour periods. At the end of 72 hours, a sample was withdrawn for the analysis of the volatile fatty acids by the method of Linke (1952), as previously described (Belasco, 1954c).

All the data shown in the tables represent individual 4-day fermentations.

Results and Discussion

Comparison of Starch, Xylan and Pectin

The effects of small amounts of starch, xylan and pectin on cellulose digestion, urea utilization and on fatty acid production were tested. The results (table 1) show that the addition of 2 gm. of starch resulted in a 33% improvement in urea utilization, a 15% increase in cellulose digestion and 30% increase in the total fatty acid concentration. The relative proportions of the individual acids remained the same.

Both xylan and pectin, when added to the system in the amount comparable to starch increased urea utilization 16% and 18%, respectively, but failed to produce any cellulolytic response. The increase in urea utilization was approximately half that obtained with starch. The substitution of 1 gm. of xylan or pectin with 1 gm. of starch gave values which were comparable to those obtained with 2 gm. of starch. No significant differences were evident in the fatty acid data between any

TABLE 1. THE COMPARATIVE EFFECTS OF CELLULOSE, STARCH, XYLAN, AND PECTIN IN THE RUMEN

Nutrient variable	Average of 24-hour data: Ammonia level	Urea utilization		Cellulose digestion		Milliequivalents of acid per 100 ml. rumen liquid: Formic	Acetic	Propionic	Butyric	Valeric	Caproic	Total
	mg.	mg.	%	gm.	%							
No urea+2 gm. starch	37	...	..	....	..	0	2.952	0.748	0.272	0.060	0.048	4.080
						0%	72.3%	18.3%	6.7%	1.5%	1.2%	100.0%
0.8 gm. urea+2 gm. starch	542	175	22	....	..	0	2.808	0.704	0.232	0.036	0.002	3.782
						0%	74.2%	18.6%	6.1%	1.0%	0.1%	100.0%
No urea+9 gm. cellulose	20	...	..	2.61	29	0.064	2.684	1.656	0.336	0.084	0.048	4.872
						1.3%	55.1%	34.0%	6.9%	1.7%	1.0%	100.0%
0.8 gm. urea+9 gm. cellulose	400	340	43	6.54	72	0.120	6.560	4.524	0.428	0.088	0.032	11.752
						1.0%	55.8%	38.5%	3.6%	0.8%	0.3%	100.0%
0.4 gm. urea+9 gm. cellulose+ 2 gm. starch	65	374	92	6.33	71	0.052	7.800	5.160	0.612	0.112	0.040	13.776
						0.4%	56.6%	37.5%	4.4%	0.8%	0.3%	100.0%
0.8 gm. urea+9 gm. cellulose+ 2 gm. starch	298	484	61	7.59	85	0.092	8.860	6.240	0.580	0.100	0.024	15.896
						0.6%	55.7%	39.3%	3.6%	0.6%	0.2%	100.0%
2 gm. xylan	370	396	50	6.40	72	0.108	8.632	5.816	0.620	0.132	0.024	15.332
						0.7%	56.3%	37.9%	4.0%	0.9%	0.2%	100.0%
2 gm. pectin	370	404	51	6.60	74	0.052	8.520	4.864	0.420	0.064	0.028	13.948
						0.4%	61.0%	34.9%	3.0%	0.5%	0.2%	100.0%
1 gm. starch+1 gm. xylan	312	458	58	7.64	85	0.032	8.856	5.792	0.532	0.092	0.024	15.328
						0.2%	57.8%	37.8%	3.4%	0.6%	0.2%	100.0%
1 gm. starch+1 gm. pectin	306	483	60	7.10	79	0.096	9.188	6.256	0.544	0.080	0.032	16.196
						0.6%	56.7%	38.6%	3.4%	0.5%	0.2%	100.0%

of the treatments. These findings would, therefore, indicate that the bacterial species which metabolize xylan and pectin do use urea nitrogen, probably not to the same extent as the amylolytic microorganisms. Furthermore, it appears that the rumen microorganisms which ferment xylan and pectin do not participate in cellulose fermentation.

Comparison of Cellulose and Starch

Varying levels of cellulose and starch, ranging from 1 to 18 gm., were compared simultaneously in the artificial rumen in regard to urea utilization, cellulose digestion, and fatty acid production. The results in table 2 demonstrate that with increases of cellulose or starch input there were corresponding increases in urea utilization and reductions in the ammonia concentration. In general, starch provided better urea nitrogen fixation than comparable levels of cellulose. The combination of 9 gm. each of starch and cellulose resulted in better urea utilization than observed with either 18 gm. of cellulose or starch. The cellulose digestion value of 64% was high in spite of the presence of a relatively large amount of starch. The total fatty acid concentration was greater with this combination than noted with 18 gm. of cellulose. The resulting fatty acids had a distribution typical of a cellulose fermentation, in spite of the starch present, with 51% acetic acid, 44% propionic acid, 3% butyric acid, and less than 1% valeric acid.

The total fatty acid concentration increased with increases in starch or cellulose input, with starch yielding more acid than comparable levels of cellulose. The total acid concentration, expressed as milliequivalents of acid per 100 ml. rumen liquid, rose from 0.81 to 14.75 and 19.50 for 18 gm. of cellulose and starch, respectively. Of particular interest was the effect of increased starch or cellulose input on the formation of the individual fatty acids. The amounts of caproic and valeric acids were small, with cellulose levels ranging from 1 to 18 gm. Their concentration never exceeded 0.5% of the total, even though their absolute concentration increased with increase of cellulose input. The absolute concentrations of butyric, propionic and acetic acids all increased with increase of cellulose input. There was a sharp rise in the relative concentration of propionic acid from 27 to 46%, and a corresponding drop in acetic acid concentration from 69 to 51% when the cellulose input was increased from 9 to 18 gm.

The fatty acid data obtained with various levels of starch show similar trends, except that starch yielded lower proportions of propionic acid and higher proportions of valeric, butyric and acetic acids than cellulose.

TABLE 2. THE EFFECT OF VARYING LEVELS OF CELLULOSE AND STARCH ON IN VITRO RUMEN ACTIVITY

Nutrient variable	Average of 24-hour data					Milliequivalents of acid per 100 ml. rumen liquid						
	Ammonia level	Urea utilization		Cellulose digestion		Formic	Acetic	Propionic	Butyric	Valeric	Caproic	Total
	mg.	mg.	%	gm.	%							
0.8 gm. urea+												
No cellulose	652	78	10	....	..	0	0.620	0.100	0.040	0.048	0	0.808
						0%	76.7%	12.4%	5.0%	5.9%	0%	100.0%
1 gm. cellulose	584	98	12	0.80	80	0	1.008	0.500	0.164	0.008	0	1.680
						0%	60.0%	29.8%	9.8%	0.4%	0%	100.0%
4 gm. cellulose	525	224	28	3.52	88	0	3.428	1.532	0.148	0.020	0.008	5.136
						0%	66.7%	29.8%	2.9%	0.4%	0.2%	100.0%
9 gm. cellulose	499	291	36	6.43	72	0	5.940	2.308	0.244	0.036	0.028	8.556
						0%	69.4%	27.0%	2.9%	0.4%	0.3%	100.0%
18 gm. cellulose	191	582	73	12.52	70	0	7.460	6.804	0.400	0.032	0.056	14.752
						0%	50.6%	46.1%	2.7%	0.2%	0.4%	100.0%
1 gm. starch	624	113	14	....	..	0	1.448	0.284	0.220	0	0	1.952
						0%	74.2%	14.5%	11.3%	0%	0%	100.0%
4 gm. starch	509	244	30	....	..	0	4.116	1.164	0.228	0.088	0.008	5.604
						0%	73.4%	20.8%	4.1%	1.6%	0.1%	100.0%
9 gm. starch	333	447	56	....	..	0	7.572	3.988	0.904	0.148	0.032	12.644
						0%	59.9%	31.5%	7.1%	1.2%	0.3%	100.0%
18 gm. starch	266	504	63	....	..	0	12.832	1.188	1.352	4.124	0	19.496
						0%	65.8%	6.1%	6.9%	21.2%	0%	100.0%
9 gm. starch+												
9 gm. cellulose	140	680	85	5.67	64	0	9.016	7.772	0.596	0.144	0.008	17.536
						0%	51.4%	44.3%	3.4%	0.8%	0.1%	100.0%

Further, with 9 and 18 gm. of starch, there was a sharp rise in valeric and butyric acid concentrations, and a correspondingly sharp reduction of propionic acid concentration at the higher starch level. Similarly, the relative concentration of acetic acid dropped from about 73% of the total acids down to approximately 60%. The same phenomenon was again observed in another test (table 3) where the same amount of starch (18 gm.) was used; the valeric acid constituted 23% of the total acids, while the propionic acid concentration was only 19%. The practical implication of these data indicates that high grain-low roughage rations could upset the fatty acid balance within the ruminant animal and possibly create nutritional disturbances related in some manner to ketosis.

In another experiment (table 3), the ratio of urea to cellulose or starch was kept constant at two different concentrations of these nutrients. It was evident again that both cellulose and starch provide environments for efficient fixation of urea nitrogen, although, as before, starch appears slightly better in this respect. The data also show that the values for the percentage urea utilization and cellulose digestion remained fairly constant when the ratio of urea and cellulose was kept constant. This appeared to be true also of urea utilization when starch was the sole energy source.

The fatty acid data point to the relationship shown in the previous experiment in that starch yielded a slightly higher concentration of total acids than cellulose and also tended to yield proportionately more valeric, butyric and less propionic acid than cellulose.

The Effect of Cellulose to Starch Ratio

The evaluation of the ratio of cellulose to starch, while maintaining the total carbohydrate concentration at 9 gm. (1%) in the system revealed that an optimum was reached where the ratio of cellulose to starch ranged from 0.5 to 2.0 (table 4). In this range, the extent of urea utilization (55%), cellulose digestion (90%) and fatty acid production reached a peak. The data show the need for some readily available carbohydrate, as previously reported by Pearson and Smith (1943) and by Arias *et al.* (1951), for optimum urea nitrogen fixation and cellulolytic activity. The data further show that the inclusion of starch resulted in greater acid production. As the starch to cellulose ratio increased, the relative concentrations of caproic, valeric and butyric acid tended to increase while that of propionic acid decreased. The relative

TABLE 3. THE EFFECT OF VARYING LEVELS OF CELLULOSE AND STARCH ON IN VITRO RUMEN ACTIVITY

Nutrient variable	Average of 24-hour data					Milliequivalents of acid per 100 ml. rumen liquid						
	Ammonia level	Urea utilization		Cellulose digestion		Formic	Acetic	Propionic	Butyric	Valeric	Caproic	Total
	mg.	mg.	%	gm.	%							
0.4 gm. urea+ 9 gm. cellulose	116	289	73	6.18	69	0.068	7.268	4.476	0.332	0.068	0	12.212
						0.6%	59.5%	36.7%	2.7%	0.5%	0%	100.0%
0.8 gm. urea+ 18 gm. cellulose	239	585	73	10.81	60	0.032	7.432	6.292	0.356	0.092	0	14.204
						0.2%	52.3%	44.3%	2.5%	0.7%	0%	100.0%
0.4 gm. urea+ 9 gm. starch	82	339	85	....	..	0.088	7.396	5.332	0.868	0.292	0	13.976
						0.6%	52.9%	38.2%	6.2%	2.1%	0%	100.0%
0.8 gm. urea+ 18 gm. starch	159	658	82	....	..	0.040	9.584	3.496	1.216	4.184	0	18.520
						0.2%	51.7%	18.9%	6.6%	22.6%	0%	100.0%

TABLE 4. THE EFFECT OF VARYING LEVELS OF CELLULOSE AND STARCH ON IN VITRO RUMEN ACTIVITY

Nutrient variable	Average of 24-hour data					Milliequivalents of acid per 100 ml. rumen liquid						
	Ammonia level	Urea utilization		Cellulose digestion		Formic	Acetic	Propionic	Butyric	Valeric	Caproic	Total
	mg.	mg.	%	gm.	%							
0.8 gm. urea+												
9 gm. cellulose	398	370	47	6.66	74	0.032	7.323	2.428	0.128	0.056	0	9.967
						0.3%	73.4%	24.4%	1.3%	0.6%	0%	100.0%
8 gm. cellulose+1 gm. starch	369	380	48	6.47	81	0.032	6.220	4.336	0.284	0.084	0.012	10.968
						0.3%	56.7%	39.5%	2.6%	0.8%	0.1%	100.0%
6 gm. cellulose+3 gm. starch	341	434	55	5.41	90	0.044	7.244	4.320	0.440	0.064	0.032	12.144
						0.4%	59.6%	35.6%	3.6%	0.5%	0.3%	100.0%
3 gm. cellulose+6 gm. starch	355	432	54	2.70	90	0.052	7.040	3.808	0.596	0.264	0.060	11.820
						0.4%	59.7%	32.2%	5.0%	2.2%	0.5%	100.0%
1 gm. cellulose+8 gm. starch	323	461	58	0.92	92	0.064	8.436	4.300	0.852	0.212	0.080	13.944
						0.5%	60.5%	30.8%	6.1%	1.5%	0.6%	100.0%
9 gm. starch	337	450	56	....	..	0.072	7.900	4.204	0.992	0.200	0.064	13.432
						0.5%	58.8%	31.3%	7.4%	1.5%	0.5%	100.0%

acetic acid concentration remained fairly constant at about 55–60% of the total acids produced.

The Comparison of Starch and Dextrose

In the final test, the influence of various levels of starch and dextrose on systems with a constant amount of cellulose was evaluated. It was noted (table 5) that as the starch input was increased, the rate of urea utilization also increased while the ammonia level showed a corresponding decrease. In contrast, increases in dextrose input, though increasing urea utilization, did not approach the effectiveness of starch. The addition of 1 gm. starch increased cellulose digestion from 52 to 79%. Additional increments of starch input up to 9 gm. did not increase cellulolytic activity further, but rather tended to decrease the improved rate of cellulose breakdown slightly from the peak of 79 to 71%. The inclusion of dextrose into the same control medium in the amounts of 1 and 3 gm. increased cellulose digestion from 52 to 77 and 80%, respectively. The addition of greater amounts of dextrose resulted in marked reductions in cellulolytic activity. At the input level of 9 gm. of dextrose, the resulting cellulose digestion (44%) was less than that in the control system without starch or dextrose (52%).

These results find precedent in the reports of Pearson and Smith (1943) and Arias *et al.* (1951). The former investigators, using undiluted rumen liquor without any added cellulose in their test systems, noted that small amounts of starch, up to 3 gm. per liter accelerated protein synthesis. Additional amounts of starch did not improve the rate of protein synthesis. Their data also indicate that the addition of starch resulted in greater protein synthesis than dextrose. Arias *et al.* (1951), using essentially the same technique as reported here, observed an accelerated rate of urea utilization with larger starch additions to a medium containing cellulose and urea. In regard to cellulose digestion, they noted a smaller increase at the lower levels of starch and an inhibition at the higher levels of starch. This difference in degree of response to starch could very well be due to differences in inoculum and in dietary regime of the source animals.

The concentration of the major fatty acids (acetic, propionic and butyric) as well as the value for total acids increased with increases in starch input. The acid distribution data revealed a slight decrease in the relative concentration of acetic acid with a corresponding increase in propionic acid. The addition of dextrose up to 3 gm. showed similar but greater increases in acid formation than corresponding amounts of

TABLE 5. THE EFFECT OF VARYING LEVELS OF CELLULOSE AND STARCH ON IN VITRO RUMEN ACTIVITY

Nutrient variable	Average of 24-hour data					Milliequivalents of acid per 100 ml. rumen liquid						
	Ammonia level	Urea utilization		Cellulose digestion		Formic	Acetic	Propionic	Butyric	Valeric	Caproic	Total
	mg.	mg.	%	gm.	%							
0.8 gm. urea+9 gm. cellulose+												
No starch	476	277	35	4.77	52	0.032	3.980	2.792	0.296	0.120	0	7.220
						0.4%	55.1%	38.7%	4.1%	1.7%	0%	100.0%
1 gm. starch	371	392	49	7.07	79	0.040	5.352	3.872	0.420	0.124	0	9.808
						0.4%	54.5%	39.5%	4.3%	1.3%	0%	100.0%
3 gm. starch	317	470	59	6.62	74	0.048	7.644	5.288	0.440	0.096	0	13.516
						0.4%	56.5%	39.1%	3.3%	0.7%	0%	100.0%
6 gm. starch	204	517	65	6.63	74	0.072	9.784	8.060	0.616	0.132	0	18.664
						0.4%	52.4%	43.2%	3.3%	0.7%	0%	100.0%
9 gm. starch	137	706	89	6.39	71	0.040	10.360	9.068	0.864	0.144	0	20.476
						0.2%	50.6%	44.3%	4.2%	0.7%	0%	100.0%
1 gm. dextrose	370	397	50	6.90	77	0.040	6.880	5.316	0.324	0.148	0	12.628
						0.3%	53.8%	42.1%	2.6%	1.2%	0%	100.0%
3 gm. dextrose	309	513	64	7.15	80	0.088	7.852	6.256	1.348	0.152	0	15.696
						0.6%	50.0%	39.8%	8.6%	1.0%	0%	100.0%
6 gm. dextrose	255	505	63	6.09	68	0.052	6.872	6.736	1.352	0.400	0	15.412
						0.3%	44.6%	43.7%	8.8%	2.6%	0%	100.0%
9 gm. dextrose	224	523	65	3.85	44	0.048	5.452	5.188	2.328	1.012	0	14.028
						0.3%	38.9%	37.0%	16.6%	7.2%	0%	100.0%
0.8 gm. urea+9 gm. dextrose	383	377	47	....	..	0	4.940	3.860	2.476	0.832	0	12.108
						0%	40.8%	31.9%	20.4%	6.9%	0%	100.0%

starch. However, beyond this level of dextrose, there was a reduction in total acid concentration which was due to reduced acetic and propionic acid formation, but which was offset slightly by increased butyric and valeric acid production. At the input level of 9 gm. dextrose, butyric and valeric acid concentrations accounted for 17 and 7%, respectively, of the total acids. Generally, dextrose in the nutrient medium yielded proportionately more valeric and butyric acids, and less acetic. The propionic acid concentration remained rather constant at 40% of the total acids.

The data presented here demonstrate the influence of the type and the amount of the energy source in the rumen on the utilization of urea, cellulose digestion and on the amount and distribution of the various volatile fatty acids elaborated. The differences in acid distribution point to different rates of acid production and/or to different metabolic pathways for the various carbohydrate substrates, which in turn may imply the action of different enzymes and/or bacterial species on these energy sources. This assumption is in agreement with the findings of McBee (1953), that all the bacteria which ferment hemicellulose could not ferment cellulose. He believes that a dynamic microbial population, changing in number and type with changes in substrates can best explain fermentative differences. Elsden (1945) noted differences in the acid distribution between the *in vitro* fermentation of cellulose and dried grass. Similarly, Gray *et al.* (1951, 1952) observed differences in the fermentation of cellulose, wheaten hay and lucerne hay. The differences in fatty acid distribution noted with the various substrates may be due to differences in the carbohydrate and nitrogen constituents of the hays.

Although the fatty acids are important to the ruminant animal as energy and metabolic intermediates, little is known of requirements of the individual acids for optimum ruminant nutrition. Such knowledge could be valuable in the formulation of rations for optimum nutrition as well as for efficient and economical management of beef and dairy animals. It is felt that the *in vitro* techniques lend themselves to rapid evaluation of feed ingredients for their contribution to the fatty acid pool.

Summary

Urea utilization by rumen microorganisms *in vitro* was dependent on the amount and type of carbohydrate used as the energy source. The extent of urea utilization was slightly greater with starch than with

cellulose. Xylan and pectin promoted urea utilization but not to the same extent as starch.

The distribution and the amount of volatile fatty acids formed were also dependent on the amount and type of carbohydrate used. Total fatty acid production was greatest with starch, followed by dextrose and then cellulose. Comparable levels of starch generally yielded proportionately more acetic, butyric and valeric acids, and less propionic acid than cellulose. Dextrose gave higher percentages of butyric and valeric acids and a lower percentage of acetic acid than starch when used in combination with cellulose.

High input levels of dextrose, when used in combination with cellulose, inhibited cellulose digestion markedly. This was accompanied by a marked increase of the relative concentrations of butyric and valeric acids and a reduction in acetic acid. Comparable high input levels of starch up to 1% of the medium in combination with 1% cellulose increased the rate of cellulose digestion and fatty acid formation.

High input levels of starch, when used as the sole carbohydrate source, resulted in a marked increase in valeric acid concentration with a corresponding decrease in propionic acid concentration.

The data point to different metabolic pathways for the various carbohydrate substrates or a dynamic microbial population changing in number and type with changes in substrate.

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THE EFFECT OF CHLORTETRACYCLINE FEEDING ON *IN VITRO* CELLULOSE DIGESTION BY RUMEN MICROORGANISMS [1, 2]

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THE growth-stimulating effect of chlortetracycline (Aureomycin) feeding to dairy calves has been demonstrated by many investigators but the effects on older cattle are not so extensive and are less conclusive. Neumann *et al.* (1951) found no beneficial effects when chlortetracycline was added to a basal fattening ration, whereas Perry *et al.* (1954) found chlortetracycline-fed steers gained faster than control animals. Bartley and associates (1953) and Rusoff *et al.* (1952) reported chlortetracycline feeding had no apparent effect on milk production. Bell *et al.* (1951) found chlortetracycline feeding to two steers decreased the apparent digestibility of crude fiber, which suggested depressed activity of cellulolytic microorganisms. Hungate *et al.* (1955) in manometric experiments demonstrated that chlortetracycline feeding altered the composition of the rumen microbial population. Methane production was diminished, probably due to inhibition of the production of intermediates. Lodge (1954) found that inoculums from chlortetracycline-fed cows consistently digested a lower percentage of purified cellulose than did those from control cows. Furthermore, when chlortetracycline was removed from the diet of two calves (Lodge, 1954), the *in vitro* cellulose digestion, which previously had been depressed, increased to apparently normal values. Inasmuch as further study of the effects of this antibiotic on cellulolytic microorganisms seemed desirable, the present investigation was undertaken to determine the effect of chlortetracycline feeding on the *in vitro* digestion by rumen microorganisms of (a) purified cellulose and (b) cellulose in a natural feed (alfalfa hay).

Experimental Procedure

Six collections of rumen liquid were made, at weekly intervals, from each of 14 lactating cows, seven of which had received approximately 240 mg. of chlortetracycline per animal daily from an early age except

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during the dry period when no antibiotic was fed. All cows were at least 4 years of age and had been lactating at least 8 months (current lactation). Therefore, chlortetracycline-fed animals had received the antibiotic continuously for a minimum of 8 months before the samples were taken. The samples were collected at approximately the same time each morning, with the aid of a stomach tube and a hand-operated stomach pump. After three collections of rumen liquid from the treated cows, chlortetracycline was removed from the diet.

The samples were strained through four layers of cheese cloth to remove the course feed particles. The liquid portion then was placed in

TABLE 1. COMPOSITION OF NUTRIENT SOLUTION[a]

Chemicals	gm. per liter
$Na_2HPO_4 \cdot H_2O$	1.2
KH_2PO_4	0.6
$NaHCO_3$	3.5
KCl	0.75
NaCl	0.75
$MgSO_4$	0.15
$FeSO_4 \cdot 7\ H_2O$	0.075
$MnSO_4 \cdot H_2O$	0.0004
$ZnSO_4 \cdot 7\ H_2O$	0.00008
$CuSO_4 \cdot 5\ H_2O$	0.002
$CoCl_2$	0.002
Urea	2.000

[a] Modified from Burroughs *et al.* (1950).

flasks in a water bath (39.5° C.) and CO_2 was bubbled into this mixture for about 2 minutes. Five hundred ml. of this liquid were centrifuged at about 900 r.p.m. for 2 minutes to sediment feed particles (and some protozoa). Four hundred ml. of the liquid were transferred to 50 ml. centrifuge tubes and centrifuged at about 2000 r.p.m. for 20 minutes.[3] The liquid portion was poured off and the sediment (containing the microorganisms) from 200 ml. of liquid was transferred into 70 ml. of mineral media (table 1) containing 700 mg. of Solka-Floc.[4] The remaining sediment from the other 200 ml. was suspended in 70 ml. of mineral medium containing 2.35 gm. of alfalfa hay (finely ground in a Wiley mill). The mineral media and rumen inoculum mixture then was diluted with distilled water to make 150 ml. and was placed in the water bath again where CO_2 was bubbled into the mixture for 10 minutes. The pH was adjusted to 7.1 with saturated Na_2CO_3 and 20 ml.

[3] International centrifuge No. 2, using 16-place head.
[4] Produced by Brown Co., Berlin, New Hampshire.

aliquots were pipetted into six 50-ml. fermentation tubes. Three tubes were retained for initial cellulose determination and the remaining tubes were placed in a 39.5° C. water bath for 24 hours. Thus, triplicate samples were employed for each determination. Carbon dioxide was bubbled through the fermentation tubes at a slow rate during the 24-hour digestion period.

To determine the cellulose content the tubes were centrifuged at 2,000 r.p.m. for one minute. The cellulose or alfalfa hay was readily sedimented and the liquid poured off. Twelve ml. of glacial acetic acid and 1.5 ml. of concentrated nitric acid were added to each tube. The tubes were placed in a boiling water bath for 20 minutes; then the contents were filtered while hot into Gooch crucibles. The sediment was washed from the tubes with 95% ethyl alcohol. The crucibles were dried in an 80° C. oven, cooled in a desiccator and weighed, then ashed in a muffle furnace at a temperature of 600° C. The crucibles were again cooled and weighed to determine the amount of cellulose in the crucibles. The difference between initial amount and that after fermentation for 24 hours represented the amount of cellulose digested. A sample from a chlortetracycline-fed cow and one from a control cow were processed concurrently.

Results and Discussion

In vitro cellulose digestion of the various combinations of substrate and inoculum are indicated in figure 1. Each point represents 21 determinations (samples from seven animals, each run in triplicate). During the first three collection periods microorganisms from control cows (cows receiving no antibiotic) digested 43.3, 42.7 and 50.0% of the purified cellulose (Solka-Floc) as compared to values of 33.8, 34.2 and 35.3%, respectively, for the first three samples from cows receiving the antibiotic. This is in accordance with the findings of Lodge (1954) who found the mean values for cows on a similar regime to be 83.5, 82.7, 87.2% for control cows and 70.1, 73.7 and 74.2%, respectively, for the chlortetracycline-fed animals. The higher cellulose digestion figures reported by Lodge probably were due largely to the greater volume of inoculum used. In the current experiment, sediment from only 200 ml. of rumen liquid was used, whereas Lodge used the microorganisms precipitated from 350 ml. of rumen liquid. When alfalfa hay was used as the substrate, cellulose digestion was 57.2, 57.3 and 62.5%, respectively, for inocula from the control animals and 43.4, 44.2 and 47.7% for inocula from animals receiving the antibiotic. The consistency of the data are very striking. Only in two instances (out of

a total of 42 comparisons) did cellulose digestion of the sample from a chlortetracycline-fed animal exceed that of the sample from the control. An analysis of variance (Snedecor, 1946) showed the difference in cellulose digestion by microorganisms from the two groups of animals to be significant at P 0.01.

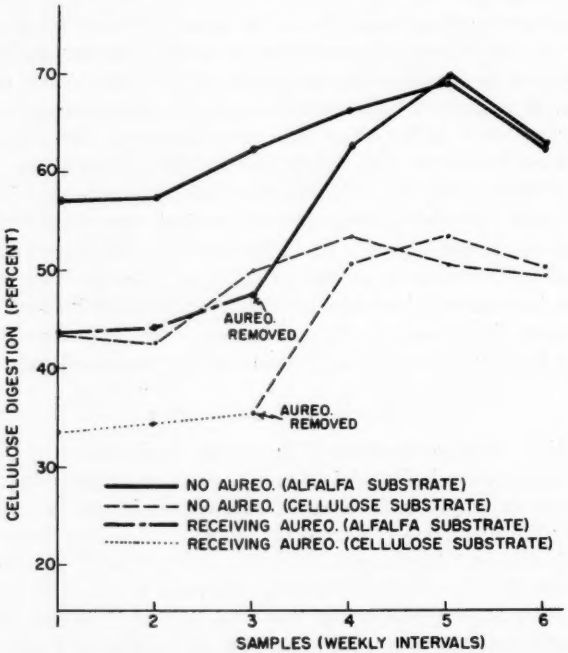

Figure 1. The effect of chlortetracycline (Aureomycin) feeding and of type of substrate, alfalfa vs. purified cellulose (Solka Floc), on *in vitro* cellulose digestion by rumen microorganisms.

During the last three collection periods, when chlortetracycline was removed from the diet of the treated cows, the percentage of cellulose digested approached that of controls with mean values for successive weeks of 53.7, 50.9 and 49.7% for controls and 50.4, 53.5 and 50.1%, respectively, for animals formerly on chlortetracycline when Solka-Floc was used as the substrate and 66.4, 69.1, 62.4% and 62.9, 69.8, 62.8%, respectively, when alfalfa hay was used as the substrate. The reason for the rapid change in cellulose digestion following removal of chlor-

tetracycline from the diet is not clear. If this results from an adjustment in types and/or number of microorganisms, the latter must be readily adaptable to such environmental changes within the rumen.

Since growth, feed utilization and milk production of dairy cattle are not adversely affected by chlortetracycline feeding, reduced *in vitro* cellulose digestion may not be indicative of the *in vivo* response. It may be that the absence of the antibiotic from the *in vitro* media resulted in the depressed cellulose digestion by microorganisms from chlortetracycline-fed cows. However, results of Lodge (1954) would indicate this not to be the case. It is possible, on the other hand, that chlortetracycline does reduce cellulose digestion in the rumen but that a concomitant favorable adjustment in efficiency of feed use occurs in the rumen or elsewhere in the gastro-intestinal tract. Further investigation is required to clarify this relationship.

Summary

Six collections (at weekly intervals) of rumen microorganisms were made from each of 14 lactating cows, seven of which had received 240 mg. of chlortetracycline per animal daily from an early age. After the collection of the first three samples from the treated cows, the antibiotic was removed from the diet.

Cellulose digestion was measured *in vitro*. During the first three collection periods microorganisms from control cows (cows receiving no chlortetracycline) digested 45.3% of a purified cellulose (Solka-Floc) as compared to 34.4% for inocula from cows receiving the antibiotic. When alfalfa hay was used as the substrate, cellulose digestion was 59% for the controls and 45.1% for cows receiving the antibiotic.

During the three subsequent collection periods when chlortetracycline was removed from the diet of the treated cows, digestion was 51.4% for controls and 51.3% for animals formerly fed the antibiotic when Solka-Floc was used as the substrate and 66.0% and 65.2%, respectively, when alfalfa hay was used as the substrate.

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AN EVALUATION OF UREA AND DICYANDIAMIDE FOR MILKING COWS [1]

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WORK reviewed by Reid (1953) indicates that urea can be utilized by dairy cows as a partial source of nitrogen. Magruder and Knodt (1954) in 1953 reported that dicyandiamide could be used as a nitrogen extender in the rations of growing dairy heifers. In this study both urea and dicyandiamide supplied 47% of the nitrogen in the grain mixture, and heifers fed these grain rations made average daily gains equal to heifers fed a grain ration containing soybean oil meal.

Lassiter *et al.* (1955) reported studies relative to the feeding of both urea and dicyandiamide as partial nitrogen replacements in the grain rations of lactating dairy cows. In this study some difficulty was encountered in keeping the protein intake of the cows near the minimum protein requirement. Even though a rather short experimental period was used (28 days), data obtained indicated that both sources of nonprotein nitrogen could be utilized for milk production.

The present paper is a report of a further study to evaluate both urea and dicyandiamide as nitrogen extenders in grain rations for milking cows.

Experimental Procedure

Twenty-four lactating dairy cows (12 Jerseys and 12 Holsteins) were used in this study. They were divided into four equal groups on the basis of milk production, body weight, age, breed and stage of lactation. All animals were past their peak of production, but none were turned dry before the termination of the experiment. Four cows were slightly more than 6 months pregnant at the end of the experiment, but they were balanced among the treatment groups.

The experimental trial was divided into two 9-week periods (Experiments I and II) with the first week of each experiment serving as an

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director. The authors express appreciation to the Allied Chemical Dye Corporation, New York, N. Y., for supplying the urea, and to the American Cyanamid Company, Lederle Laboratories Division, Pearl River, N. Y. for supplying the dicyandiamide.

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adjustment period. The second experimental period followed immediately after the first, and the same cows were used in each trial with the exception of one that was replaced at the beginning of the second experiment owing to low production. The feeding order of the cows remained the same in both trials, e.g., cows in group 1 received the basal ration during both experiments.

The four experimental grain rations used in Experiment I along with their chemical composition are shown in table 1. Approximately one-

TABLE 1. COMPOSITION OF EXPERIMENTAL GRAIN MIXTURES (EXPERIMENT I)

Ingredient	Mixture 1	2	3	4
	lb.	lb.	lb	lb.
Corn cob meal	750	900	900	900
Wheat bran	150	150	150	150
Soybean oil meal	100	8.6	8.6	8.6
Urea (275% protein eq.)	...	...	13.7	...
Dicyandiamide (416% protein eq.)	...	...	...	9.6
Iodized salt	10	10	10	10
Steamed bone meal	10	10	10	10
Total	1020.0	1078.6	1092.3	1088.2
	Chemical composition			
	%	%	%	%
Dry matter	88.1	88.7	89.5	91.0
Crude protein	13.4	9.0	12.7	13.2
Ether extract	3.3	3.4	3.4	3.7
Crude fiber	7.3	8.5	7.7	8.8
Ash	4.1	3.1	3.2	3.2
Nitrogen-free extract	60.0	64.7	62.5	62.1

third of the crude protein in rations 1, 3, and 4 was supplied by soybean oil meal, urea or dicyandiamide, respectively. Ration 2 was essentially the same as the other rations except it contained about one-third less crude protein and acted as a negative control. The grain rations fed during Experiment II are not shown, but they were essentially the same as fed during Experiment I except the crude protein content of rations 1, 3 and 4 was increased from about 13.0% to slightly more than 15.0%. The protein content of ration 2, the negative control, was increased from 9.0% in Experiment I to 10.7% in Experiment II. The dicyandiamide used in these studies contained 416% protein equivalent.

The roughage portion of the rations in Experiment I was composed of alfalfa hay and corn silage, fed at a daily rate of 1 lb. of hay and 3 lb. of silage for each 100 lb. of body weight. During Experiment II, the alfalfa hay was replaced by a medium-quality mixed hay (one-fourth red clover and three-fourths timothy). The rate of concentrate feeding at the start of Experiment I was based on Morrison (1948) minimum standards. Adjustments in grain feeding at the end of each 4-week period were based on the average percentage in milk production during the preceding 4 weeks. The cows were fed at a rate approximating 110% of these total digestible nutrient standards. When alfalfa hay was fed difficulty was encountered with low-producing cows near the end of Experiment I owing to their failure to consume enough grain to meet their TDN requirements and yet not overfeed on protein. This was the basis for changing to the feeding of timothy-red clover hay during Experiment II. During the second experiment the cows on rations 1, 3 and 4 were fed concentrates at a rate to supply only minimum protein requirements, and cows on ration 2 were fed protein at a rate 80–85% of Morrison's (1948) minimum protein standards.

Daily milk weights were recorded, and composite milk samples were taken 2 days each week for butterfat analysis. Body weights were obtained prior to and and at the end of the preliminary period and twice during each experiment. Venous blood and milk samples were taken every 2 weeks and analyzed for urea nitrogen by the method of Van Slyke and Cullen (1916). Digestion trials were conducted using chromic oxide as the indicator twice during each experiment with two cows from each group. After a 10-day preliminary period fecal "grab" samples were taken for 5 consecutive days, at 6:00 a.m. and 4:00 p.m. Chromic oxide determinations were made according to the method outlined by Gehrke and Baker (1954) which is a modification of the method published by Bolin (1952). All other analyses were conducted according to accepted A.O.A.C. (1950) methods.

Results and Discussion

Milk Production

Data relative to the milk production of the four experimental groups for both experiments are shown in table 2. Satisfactory milk yields were obtained on all rations in both experiments, including the production of cows fed ration 2, the low-protein ration. A lower milk production from cows fed ration 2 was expected since these cows were fed inadequate

amounts of protein, especially during Experiment II, based on present feeding standards (Morrison, 1948). Since the milk production of cows fed the low-protein ration (ration 2) was comparable to the milk production of the cows fed the other rations, it is difficult to evaluate properly the two sources of non-protein nitrogen. The cows fed rations 2 and 4, the low-protein ration and the ration containing dicyandiamide, did decline in milk production more than cows fed rations 1 and 4, rations containing soybean oil meal and urea, but these differences were not statistically significant (Snedecor, 1946). The cows fed a ration containing dicyandiamide (ration 4) declined in milk production the

TABLE 2. AVERAGE PRODUCTION OF 4% FAT-CORRECTED MILK AND THE AVERAGE DECLINE IN MILK PRODUCTION (LB. PER COW PER DAY); AVERAGE CHANGE IN BODY WEIGHT (LB. PER COW PER TRIAL)

Ration	Av. daily F. C. M.	Decline in F. C. M.	Body weight change
	lb.	%	lb.
	Experiment I		
1. Soybean oil meal	27.6	16.6	−50
2. Low protein	28.2	19.2	−59
3. Urea	26.8	17.2	−69
4. Dicyandiamide	25.7	22.6	−54
	Experiment II		
1. Soybean oil meal	21.3	16.5	+44
2. Low protein	22.2	16.5	+ 7
3. Urea	22.8	9.1	+22
4. Dicyandiamide	22.1	25.0	+28

most of any group in both experiments; especially was this true in Experiment II when the cows were fed protein at the minimum level. It is difficult to explain why cows fed ration 2 did not decline in milk production more than those fed the other rations, but such factors as the level of milk production and length of the experimental period may have been responsible. It is quite possible that if the ration had been fed over a longer period of time, a statistically significant decline in milk production of cows fed this ration would have occurred. It should be pointed out that the rate of TDN feeding was at a level of about 110% of Morrison's (1948) recommended allowances, which means that the protein in the ration was available for protein metabolism and probably very little if any was being used for energy purposes.

Body Weights

The effect of the various rations on change in body weight of cows for both experiments is shown in table 2. All cows, regardless of the ration being fed, lost weight during Experiment I and gained weight during Experiment II. None of the differences between rations for body weight changes were statistically significant. The cows probably lost weight during Experiment I owing to the high plane of nutrition being followed before they went on experiment. The cows fed ration 2 during Experiment II gained the least weight of any group, but this was largely due to two cows that lost weight, probably caused by their excessive hay refusals. None of the experimental rations appeared to have a marked influence on body weight changes.

TABLE 3. AVERAGE DAILY FEED CONSUMPTION OF COWS DURING EXPERIMENT I AND II

Ration	Experiment I			Experiment II		
	Grain	Hay	Silage	Grain	Hay	Silage
	lb.	lb.	lb.	lb.	lb.	lb.
1	8.5	10.7	32.5	6.1	10.7	32.5
2	8.6	10.3	31.0	6.6	9.6	31.0
3	8.4	10.8	32.5	6.2	10.6	32.4
4	8.3	11.1	33.5	6.5	11.1	33.4

Feed Consumption

Feed consumption data are presented in table 3. Although there were no appreciable differences between groups in the amounts of feed consumed, it appeared that the concentrate mixtures containing the nonprotein nitrogen compounds were slightly less palatable than the other grain mixtures. In these studies, however, palatability was not a serious problem. Only one animal on each of the urea and dicyandiamide grain rations showed any signs of feed refusals.

Blood and Milk Urea Nitrogen Studies

Data obtained concerning blood and milk urea nitrogen values are presented in table 4. It was thought that a study of these values would yield information concerning the utilization of nitrogen being fed. Archibald (1946) observed that the level of protein in the ration influenced the blood and milk urea nitrogen values. In Experiment I the blood urea nitrogen values for cows fed rations 1 and 3 were significantly higher

than those for cows fed rations 2 and 4. Cows fed rations 1, 3 and 4 had significantly higher levels of urea nitrogen in their milk than did cows fed ration 2. The milk values for cows fed ration 1 were significantly higher than those fed ration 3 or 4 and the milk of cows fed ration 3 contained significantly more urea nitrogen than did cows fed ration 4. These data clearly demonstrate that the level of protein fed influenced the blood and milk urea nitrogen levels. These data also indicate that nitrogen supplied by dicyandiamide was not being utilized to the extent of nitrogen supplied by soybean oil meal or urea.

TABLE 4. AVERAGE UREA NITROGEN LEVELS IN BLOOD AND MILK (MG. N PER 100 ML.)

	Experiment I		Experiment II	
Ration	Blood	Milk	Blocd	Milk
1	13.5	12.4	9.0	8.9
2	12.3	9.9	8.1	7.9
3	14.0	11.4	10.0	8.6
4	12.0	10.6	8.1	7.8

The blood and milk urea nitrogen data for Experiment II were essentially the same as for Experiment I except all values were lower. Cows fed rations 1 and 3 showed significantly higher blood urea nitrogen levels than animals receiving rations 2 and 4. The differences between groups in regard to milk urea nitrogen values were not significant, although the values for cows fed rations 2 and 4 were lower than the other two rations.

Digestibility Studies

The effect of the various rations on the digestibility of feed nutrients is shown in table 5. It has been shown by Thompson *et al.* (1952) that a subnormal level of protein in the ration of milking cows not only fails to meet the animals' requirements, but also exerts a depressing influence upon the digestibility of feed nutrients. Pope *et al.* (1950) reported that the addition of urea to a low-protein ration improved the utilization of the entire ration. The coefficients shown in table 5 are for the entire ration fed, not just the concentrate mixtures. None of the differences between rations with any nutrient were statistically significant; however, the differences in dry matter digestibility in Experiment II approached significance. In Experiment I, the crude fiber coefficients of digestibility for the two rations containing the non-protein nitrogen

compounds, rations 3 and 4, were lower than for the other rations. The addition of the non-protein nitrogen compounds in Experiment II to the low-protein ration, ration 2, tended to increase the utilization of all nutrients except nitrogen-free extract.

Summary

Twenty-four lactating dairy cows were used in two 56-day experiments to further evaluate the use of urea and dicyandiamide in the concentrate mixture of milking cows. Urea and dicyandiamide replaced approximately one-third of the nitrogen in a basal ration when soybean oil meal was used as the source of nitrogen. A fourth concentrate ration was included in each experiment which contained one-third less protein than the other rations. Alfalfa hay and corn silage were fed as roughages in Experiment I, and corn silage and timothy-red clover hay were used during Experiment II. During Experiment II all cows were fed below the minimum recommended amount of protein.

No significant differences in milk production in either experiment between the four rations were observed. The cows fed the low-protein ration and the ration containing dicyandiamide declined in milk production more than cows fed rations containing either soybean oil meal or urea. No differences in body weight changes or feed consumption were observed.

TABLE 5. AVERAGE COEFFICIENTS OF APPARENT DIGESTIBILITY OF THE EXPERIMENTAL RATIONS (%) [a]

Nutrient	Ration 1	2	3	4
		Experiment I		
Dry matter	58.0	57.0	52.7	55.5
Crude protein	62.2	56.6	57.0	59.4
Ether extract	70.2	69.3	70.7	71.5
Crude fiber	42.8	45.7	33.9	39.3
Nitrogen-free extract	68.4	67.6	65.0	68.5
		Experiment II		
Dry matter	60.4	53.3	54.4	55.6
Crude protein	52.7	38.8	47.6	45.8
Ether extract	54.5	40.1	66.7	75.0
Crude fiber	53.4	43.6	46.5	49.3
Nitrogen-free extract	68.6	67.8	67.3	68.0

[a] Coefficients for entire ration.

The ration being fed appeared to influence the level of urea nitrogen in both the blood and milk. Cows fed the low-protein and dicyandiamide rations tended to have lower levels of urea nitrogen in both the blood and milk than cows fed the rations containing either soybean oil meal or urea. No significant differences were observed in the digestibility of feed nutrients of the various rations. Evidence was obtained in Experiment II that the addition of non-protein nitrogen to the low-protein ration caused slightly better average utilization of the feed nutrients, but the differences were not significant.

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FACTORS CONTRIBUTING TO THE DIFFERENCE IN DRESSING PERCENTAGE BETWEEN HEREFORD AND BRAHMAN × HEREFORD STEERS

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THE dressing percentage of cattle is an economic factor of great importance. The chilled carcass yield as a percentage of the sale weight is one of the factors which has great influence on carcass costs. A decrease in yield is expected to accompany decrease in slaughter grade, and some market classes are expected to yield much higher than others.

Black *et al.* (1934) reported that steers which were about half Brahman had dressing percentages between 2 and 4% higher than non-Brahmans. The latter were Herefords and Shorthorns. The average difference was 2.4%, which is in close agreement with the difference of 2.7% reported by Butler *et al.* (1955). Carroll (1955) found that Brahman × Hereford crossbred steers and heifers dressed 2.2% higher that Herefords after about 200 days drylot feeding. Data were obtained from 14 animals of each breed and sex.

Hubert *et al.* (1955) reported that the dressing percentage was about the same in a comparison of Brahman × Hereford steers and heifers with Herefords. Their data, however, showed that 18 Brahman steers yielded 58.8 compared with 57.0% for the same number of Herefords. Six Brahman × Hereford heifers yielded 55.8 compared to 55.5% for five Herefords. Their reported range of 7% among individual cattle seems high. Marketing conditions were not described, but the dressing percentages were about 3% lower than reported by Butler *et al.* (1955), indicating that the cattle probably carried more fill.

Black *et al.* (1934) tested about 300 steers over a three year period. About half of these steers were crossbreds, Brahman × Herefords and Shorthorns, and the other half were of Hereford and Shorthorn breeding. Their conclusion that "the higher dressing percentage of cattle of Brahman breeding is indisputable" seems to be substantiated by the results of Butler *et al.* (1955) based on 59 Hereford and 90 Brahman × Hereford steers and by Carroll *et al.* (1955).

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Recent figures obtained by Jones *et al.* (1955) on 15 Brangus and 56 Hereford steers treated alike experimentally and fed in drylot about 10 months on rations containing 10 to 14 lb. of concentrates are not in agreement with the general conclusion that Brahman breeding increases dressing percentage. The Brangus steers had a pay weight of 931 lb. at El Paso, shrank 5.36% en route by train to Fort Worth, and yielded 62.61% based on pay weight and 66.15 based on arrival weight and warm carcass. The El Paso pay weight for the Herefords was 963 lb., shipping shrinkage 4.49% and yield 65.64% on the basis of pay weight and 68.73 on the basis of arrival weight and warm carcass. Gains in the feedlot and carcass grades also favored the Herefords.

The factors causing the yield advantage of part Brahman cattle were investigated to the extent possible in a commercial packinghouse by Black *et al.* (1934). More detailed investigation seemed desirable, and was accomplished on the cattle produced on an S-10 Southern Regional Beef Cattle Breeding Project as described by Warwick and Cartwright (1955), at Texas Agricultural Experiment Station Substation 23, and slaughtered and processed in the Meats Laboratory at College Station, Texas.

Procedure

The steers were weighed individually from the feedlot and trucked about 110 miles from McGregor to College Station to arrive at about 5 p.m. They were weighed again immediately after unloading, and held in a paved pen with water available but no feed. All steers were weighed again at about seven the following morning, and slaughtered before noon. About ten steers were slaughtered each of four consecutive days.

The slaughter procedure was essentially as recommended by Deans (1951). The digestive tract was trimmed free of caul and ruffle fat, and divided into rumen and reticulum, omasum and abomasum, and intestines. After weighing, the sections of the tract were emptied and reweighed. The paunch sections were washed after emptying and before weighing, but the intestines were not. All dressoff items thought to have an important effect on dressing percentage were weighed, except blood.

Eighteen Herefords and 20 Brahman $\times$ Hereford steers out of Hereford cows were slaughtered. The steers were selected at random within sire groups, and were split evenly between those having received a "growing" ration of approximately 35% concentrates and those having received a "fattening" ration of approximately 65% concentrates. Four

progeny each of five Brahman bulls and three Hereford bulls and six progeny of one Hereford bull made up the slaughter test group. The rate of gain test was for a period of 140 days following a 14-day adjustment period, and the steers were kept on the same experimental rations until slaughter 15 to 18 days after the gain test was completed. A total of 47 Hereford and 50 Brahman × Hereford steers were included in the gain test. Drylot feeding was started when the calves were weaned. At slaughter the steers averaged 14 months of age, and ranged from 13 to 15 months. Herefords averaged 743.5 lb. at slaughter and crossbreds 782.2 lb.

Results and Discussion

Table 1 shows the mean values obtained, and gives some data obtained by Black *et al.* (1934) for the same breeds fed a similar period of time in dry lot.

The crossbred steers were noticeably more excitable, a characteristic noted, also, by Black *et al.* (1934), Carroll *et al.* (1955), and Hubert *et al.* (1955). This trait has been thought to cause more shipping shrinkage and to account for some of the increased yield of cattle carrying Brahman blood. This observation may well hold true where the cattle are allowed feed after shipping before sale as commonly practiced at stockyards. The fact that the Hereford steers showed more shipping shrinkage and still had more fill at slaughter emphasizes the observations of Black *et al.* (1934) that the crossbred Brahman steers habitually eat less at one time but eat more frequently in the feedlot. The significant difference in weight of the empty digestive tracts also agrees with their belief that the crossbred steers may have smaller digestive tracts.

The difference in yield of 2.73% was accounted for largely by the 1.69% difference in total fill added to the 0.68% difference in weight of the empty tract. Nearly all of the increased fill of the Hereford steers was in the rumen and reticulum.

The breed × feed interaction was highly significant for total fill because the crossbred steers showed 6.28% on the low concentrate ration and 6.03% on the high compared to 8.70 and 6.99% for the Herefords. There was little difference in average gain of all the steers between the two rations, with Herefords gaining 2.24 lb. per day on the high concentrate ration and 2.19 on the low, and crossbreds 2.02 on the high and 2.13 on the low. The difference between feed groups of Herefords apparently would have been greater if they had carried

equal fill. The gain response and carcass grades showed that the Herefords made better utilization of the high concentrate ration than the crossbreds, but that the crossbreds performed about equally well with low or high concentrates.

TABLE 1. COMPARISON OF SHIPPING SHRINKAGES AND PERCENTAGES OF DRESSOFF ITEMS BETWEEN HEREFORD AND BRAHMAN × HEREFORD STEERS

Item	Hereford	Brahman × Hereford	Data of Black *et al.* (1934) Hereford	Data of Black *et al.* (1934) Brahman × Hereford
	%	%	%	%
Shrink in transit[a]	2.63	2.53	.....	.....
Shrink overnight[a]	4.49	2.95**	.....	.....
Head, tongue in	3.33	3.19	3.26	3.07
Removed shanks and feet	1.92	1.95	1.69	1.68
Liver, gall on	1.44	1.35*	1.30[b]	1.24[b]
Heart, trimmed	.35	.34	.42[c]	.39[c]
Pluck, less heart	1.40	1.33	1.05[d]	.97[d]
Hide	8.36	8.13	8.3	8.0
Total stomach, full	10.09	8.22**	11.4	8.5
Rumen and reticulum, full	7.46	6.10**	.....	.....
Rumen and reticulum, empty	1.81	1.70**	.....	.....
Contents of rumen and reticulum	5.65	4.40**	.....	.....
Omasum and abomasum, full	2.63	2.12**	.....	.....
Omasum and abomasum, empty	1.25	1.16	.....	.....
Contents of omasum and abomasum	1.38	.96**	.....	.....
Intestines full, fat off	3.78	3.28**	4.8	4.1
Intestines, empty	2.97	2.48**	.....	.....
Intestinal contents	.81	.79	.....	.....
Total fill	7.84	6.15**	.....	.....
Digestive tract, empty	6.02	5.34**	.....	.....
Caul and ruffle fat	2.56	2.38	1.60	1.76
Dressing percent, chilled	60.25	62.98**	55.8[e]	58.3[e]

* Significant at .05 level.
** Significant at .01 level.
[a] Based on feedlot weight.
[b] Livers were weighed with gall removed.
[c] Untrimmed hearts.
[d] Did not include esophagus.
[e] Based on final feedlot weight and warm carcass weight.

Only two of the crossbred steers graded choice (table 2), and the average for the low concentrate ration was average good. Those on the high concentrate ration fell in the same category with only a fractional advantage. Two carcasses from each group graded commercial.

Six of the Hereford steers on the high concentrate ration graded Choice and 3 graded good but high good was the highest rating for those on the growing ration, with 2 rated average good and one average commercial. The one-half grade advantage of the Herefords on the high concentrate ration compared to those on the low concentrate allowed two-thirds of them to cross the economically important good-choice borderline.

An effect of sires within breeds on percentage of dressoff items also was significant at the .05 level for total fill, weight of the total stomach full, and weight of the full omasum and abomasum.

TABLE 2. DAILY GAINS, SHRINKAGES, DRESSING PERCENTAGES, AND GRADES

	Hereford steers		Brahman × Hereford steers	
Concentrate level	High	Low	High	Low
Number	9	9	10	10
Average daily gain, lb.	2.24	2.19	2.02	2.13
Shrink in transit, %	2.60	2.66	2.41	2.66
Shrink overnight, %	4.60	4.37	2.31	3.59
Dressing percentage	60.88	59.63	62.92	63.04
Grades				
Choice	6	0	2	0
Good	3	8	6	8
Commercial	0	1	2	2

Summary

Eighteen Hereford and 20 Brahman × Hereford steers from Hereford dams raised under the same conditions and fed alike on a growing ration and a fattening ration were slaughtered and compared. Dressoff items were weighed in an attempt to account for the 2 to 3% advantage in dressing percentage of the crossbred Brahman steers.

Shrinkage in trucking about 110 miles was about the same for the two groups, but the Hereford steers showed about 1.5% more shrinkage on overnight stand before slaughter when water but no feed was available.

Based on slaughter weight and chilled carcass weight the dressing percentage was 60.25 for the Herefords and 62.98 for the crossbreds. The contents of the digestive tract amounted to 7.84% of the slaughter weight of the Herefords, and 6.15% for the crossbreds. Most of this

difference was found in the paunch. The empty digestive tract was 6.02% of the slaughter weight for the Herefords, and 5.34% for the crossbreds. Other dressoff items exerted little influence on the 2 to 3% yield difference noted consistently over a four year period for the two breeds.

Hereford steers made better use of the high concentrate ration as shown by gains and grades when compared to Hereford steers on the low concentrate ration. Ration had little effect on gains or grades of the crossbred Brahman × Hereford steers.

Sires within breeds caused a significant (.05 level) difference in total fill in the digestive tract, which suggests inheritance of digestive capacity or eating habits or both.

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HERITABILITY OF WEANING GRADE IN RANGE BEEF CATTLE

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IN a range beef herd a calf's performance at weaning is doubly important in that it is a measure of its inherited growth potential and is also a measure of its dam's productivity. Weaning weight and weaning grade or score are two of the common measures of such performance. There are differences of opinion as to the importance of weaning grade. A rational basis for deciding such an issue is a knowledge of the heritability and relative economic importance of weaning grade as compared with other selection criteria that might be used.

This study presents estimates of heritability of weaning grade in a range Hereford herd. The number of estimates of heritability of weaning grade in range beef cattle reported in the literature is not large. Knapp and Clark (1950) estimated the heritability of weaning grade of range Hereford cattle to be 28%. Koger and Knox (1952) obtained heritability estimates of 50% and 30% in a range herd of Aberdeen Angus and 23% and 24% in a range herd of Herefords; in each case the former estimate is based on an offspring dam regression coefficient while the latter is based on a paternal half sib correlation.

Material

The data analyzed in this study are the weaning grades of 577 calves produced by two grade Hereford herds maintained by the University of California at the San Joaquin Experimental Range (operated by U. S. Forest Service) in the foothills of the Sierras east of Madera, California. The weaning grades were gathered over an 11-year period from 1937 to 1947. A detailed description of the constitution and management of these herds has been presented by Wagnon, Guilbert, and Hart (1942) and Rollins and Wagnon (1956). A brief summation of the management of these herds is as follows:

In June 1935, 70 head of medium to good grade 2-year-old bred Hereford heifers were brought to the Range. They were run together and re-

[1] San Joaquin Experimental Range, O'Neals, California.

[2] In co-operation with the U.S.D.A. under Western Regional Project W-1 on beef cattle breeding research.

ceived supplemental feed during their first calving, which occurred during the ensuing fall and winter. In January 1936, the heifers and their calves were divided into two comparable groups, A and B. Herd A received supplements during the late summer, fall, and winter until adequate new green forage became available, while Herd B received no supplements except in emergency to stop death losses. From the advent of new forage growth until after the calves were weaned the fore part of July neither herd received supplements. The dry forage of the Range

TABLE 1. GRADING GUIDE FOR BEEF CATTLE

Grade designation	Numerical value	Description		
			Market cattle	
		Breeding cattle	Feeders	Slaughter
	100(1+)	The top of the grade represents outstanding animals in strong competition. The middle and lower end of the grade represent excellent breeding animals from standpoint of type, conformation, quality, character, capable of making a good showing in strong competition.	Strictly fancy or select.	Top prime, capable of creditable showing in strong, single or carlot competition.
Grade 1	97(1)			
	94(1—)			
	91(2+)	Cows in grade 2 are good enough to retain for breeding test in purebred herds. This is a practical top for commercial cattle. The top of grade 2 represents the best of range bulls; the lower end of herd bulls. Cattle in this grade are well down the line or out of the money in strong competition.	Top choice	Prime
Grade 2	88(2)		Choice	to
	85(2—)		Low choice	Low prime
	82(3+)	Cows usually should be culled from purbred herds; good commercial cattle; bulls rarely capable of making much improvement except of very plain cattle.	Top good	Top choice
Grade 3	79(3)		Good	to
	76(3—)		Low good	Low choice
	73(4+)	Plain, upstanding, thin-fleshed, slow maturing cattle, lacking in quality and character and having serious defects of conformation. Should be culled from commercial herds.	Top medium	Good
Grade 4	70(4)		Medium	Commercial
	67(4—)		Low medium	Low commercial

was found to be deficient in crude protein and at times in phosphorus. Starting with the germination of the new forage crop and until the new forage growth became adequate in abundance there was also a deficiency of total energy intake. The A Herd cows received supplements during the period of deficient nutrition in amounts judged necessary to meet the deficiencies occurring.

The breeding of the two herds were similar in that the same bulls from the University's registered Hereford herd were used on each herd.

The grading system used was that developed by Guilbert (1951). It is summarized in table 1. For purposes of statistical analysis a unit

value was assigned to each one-third of a grade difference. The lowest recorded grade "4—" was assigned a value 1, "4" a value 2, etc.

With the exception of 1939 the grading was done at weaning time each year by the same person. Steer and heifer calves from both the A and B herds were graded in an indiscriminate group. In 1939 the calves were graded by a different person four to five weeks after weaning. The 1939 grades averaged much lower than those of the other years. They were omitted from the analysis in view of the different circumstances under which they were obtained.

TABLE 2. REGRESSION COEFFICIENTS OF WEANING GRADE ON WEANING WEIGHT AND THE CORRESPONDING CORRELATIONS

	Regression coefficient of weaning grade on weaning wt.	Correlation of weaning grade and weaning wt.
Between years	b_1=.0014	r_1=.11
Within years	b_2=.0083	r_2=.42
Between sex within years	b_3=.0061	r_3=.37
Within sex	b_4=.0083	r_4=.42

Tests of significance

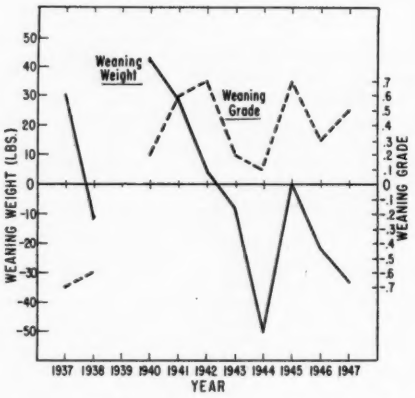
	F values		
		Level of rejection of hypothesis	
Hypothesis tested	Sample F	F .05	F .01
b_4=0	107.36	3.86	6.69
b_4=b_3	.17	3.86	6.69
b_2=b_1	13.60	3.86	6.69

The relation between weaning weight and weaning grade was studied. The results are set forth in table 2 and figure 1.

Heifers averaged slightly lower in grade than steers and since the hypothesis b_4=b_3 was not rejected (see table 2) it follows that the sex difference in grade was largely a result of a difference in weight. That is to say, to the extent that the sex difference in weaning grade was due to factors other than a sex difference in weaning weight b_4 should differ from b_3. However the fact that the b_3 estimate is smaller than the b_4 estimate may be further evidence for the widely accepted belief that heifers mature earlier than steers. Heifers averaged 6.59 in grade

and 436 lb. in weight while the corresponding averages for steers were 6.72 and 460 lb.

Since the hypothesis $b_4=b_3$ was not rejected b_2 becomes the regression to use within years. The hypothesis $b_1=b_2$ then was tested and rejected. The rejection of the latter hypothesis implies that the relation between weaning grade and weaning weight for yearly averages is not the same as the corresponding relationship within years. A comparison of the time trends for weaning grade and weaning weight in figure 1



G_1	G_2	G_3	Year	W.G.	W.W.(lb.)
			1937	− .7	29
			1938	− .6	− 12
			*1939	− 2.1	15
			1940	.2	43
			1941	.6	29
			1942	.7	4
			1943	.2	− 8
			1944	.1	− 50
			1945	.7	0
			1946	.3	− 22
			1947	.5	− 33

G_1 The offspring of Foundation cows.

G_2 The offspring of replacement cows selected from G_1.

G_3 The offspring of replacement cows selected from G_2.

An arrow indicates the years over which a generation was reproduced.

* Omitted from analysis. See comment in text.

Figure 1. Yearly deviations of weaning grade and weaning weight from their respective means.

indicates that improvement in weaning grade through selection did occur but is confounded with the effects of yearly fluctuations in weaning weight. The decline in weaning weights during the latter years of the experiment was due to poor range forage resulting from adverse climatic conditions.

In view of this the data were subdivided according to generation and herd. Distributions of weaning grades for herds and generations are presented in table 3. The heritability of weaning grade was estimated for each group on a paternal half sib basis. This material is presented in table 4. Are the six estimates in table 4 from the same population? By generation the differences show no consistent trend. By herd those of herd A are consistently higher than those of Herd B. The estimates were pooled by herds and 95% confidence limits were calculated. For

TABLE 3. DISTRIBUTIONS OF WEANING GRADES FOR HERDS AND GENERATIONS

Generation	Herd	Grades										
		1	1−	2+	2	2−	3+	3	3−	4+	4	4−
G_1: Offspring of foundation cows	A	1		2	21	14	19	13	3		1	
	B	2			18	16	12	15	4	2	4	
G_2: Offspring of replacement cows selected from G_1	A	1	1	15	56	48	18	6	1			
	B	1		5	28	38	19	24				
G_3: Offspring of replacement cows selected from G_2	A			6	16	20	12	3				
	B				11	16	11	3				

Herd A the estimate is 67% with a lower confidence limit of 23%. For Herd B it is 8% with an upper confidence limit of 55%. The extent of overlap of the confidence limits does not suggest a significant difference between the two herd estimates. Pooling all six estimates gives a heritability estimate of 36% which is considered the best estimate these data yield on the basis of the paternal half sib method.

The data included weaning grades of the replacement cows and weaning or yearling grades of the bulls used in grading up the herds. Using the heritability estimate of 36% and calculating the selection differentials of the parents that produced generations G_2 and G_3 the expected improvement in weaning grade was calculated. In Figure 2 this expected improvement in weaning grade is compared with the observed improvement. The expected improvement per generation is given by the formula $\triangle G = g^2 \triangle P$ where $\triangle G$ is the expected improvement, g^2 the heritability and $\triangle P$ the selection differential.

Discussion

The correlation between weaning grade and weaning weight within years is .42. The square of this is .18 thus 18% of the variance in

TABLE 4. HERITABILITY ESTIMATES OF WEANING GRADE

Generation	Herd A		Herd B	
G_1: The offspring of foundation cows	No. of offspring	74	No. of offspring	73
	No. of sires	14	No. of sires	15
	Heritability	.40	Heritability	.36
G_2: The offspring of replacement cows selected from G_1	No. of offspring	147	No. of offspring	115
	No. of sires	16	No. of sires	16
	Heritability	.68	Heritability	−.32
G_3: The offspring of replacement cows selected from G_2	No. of offspring	57	No. of offspring	41
	No. of sires	13	No. of sires	11
	Heritability	1.06	Heritability	.32

weaning grade within years was due to variation in weaning weight. The regression of weaning grade on weaning weight within years explains the lower average weaning grade of Herd B compared with that of Herd A. The weaning grades and weaning weights of Herd A calves averaged .6 and 48 lb. higher respectively than those of Herd B calves.

Since the weaning grade heritability estimate of 36% was obtained

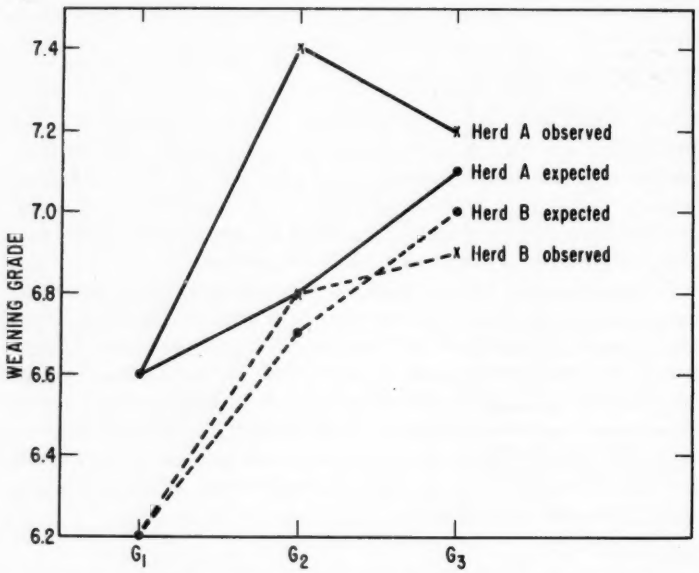


Figure 2. Expected and observed change in weaning grade during two generations of selection in Herds A and B.

from within generation data, some evidence of its accuracy can be obtained by comparing the change in average weaning grade over successive generations with the expected change based on the formula $\triangle G = g^2 \triangle P$ where $\triangle G$ is the expected change in one generation, g^2 the heritability and $\triangle P$ the selection differential. Figure 2 presents this comparison. In the case of Herd A the agreement between the observed and expected weaning grade in G2, the first generation resulting from selection is poor. Over a two generation span the agreement is good. The poor agreement in G2 does not seem to be related to yearly fluctuations in weaning weight. In the case of Herd B the agreement is good in

both G2 and G3. Comparisons of the average weaning grades of G2 with those of G3 in both herds A and B reflect the effect of the lighter weaning weights occurring during the latter five years of the experiment as shown in Figure 1.

Over the two generation span progress in Herd A was 20% greater than expected while progress in Herd B was 12% less than expected. This along with the fact that the heritability estimates within generations were consistently higher for Herd A than for Herd B suggests that heritability of weaning grade may be slightly higher under the more adequate nutritive conditions of Herd A than under the poorer nutritive conditions of Herd B. The data however do not afford sufficient evidence of any difference of statistical significance between the estimates for Herd A and Herd B. The paternal half sib estimates for Herd A and for Herd B are 67% and 8% respectively. Use of these heritability estimates to compute expected gains results in much poorer agreement with observed rates of gain than when the average value of the heritability estimates for the two herds is used.

If $\triangle G$, now standing for the observed change in weaning grade per generation, and $\triangle P$, the selection differential are each averaged over the two generations of selection, then $g^2=\frac{\triangle G}{\triangle P}$ gives the following heritability estimates of 42% for Herd A and 29% for Herd B. An unweighed average of these is 36%, the same estimate obtained from pooling the paternal half sib within generation within herd heritability estimates.

These heritability estimates fall within the range of those reported for range beef cattle by Knapp and Clark (1950) and Koger and Knox (1952). The estimates reported in these two studies are 23%, 24%, 28%, 30% and 50%.

Summary

The heritability of weaning grade has been estimated in two experimental range herds, A and B, of similar breeding that were managed alike except that in Herd A cows were supplemented during the fall and winter when the range was nutritively deficient while the cows in Herd B were not supplemented. On the basis of paternal half sib relationships heritability estimates were made within each of three generations for each herd. The estimates for Herd A were consistently higher than those for Herd B. However, the herd difference in the estimates

was not statistically significant. The average value of the heritability estimated for the two herds was 36%.

On the basis of selection experiments extending over two generations heritability of weaning grade in Herd A was estimated to be 42% and in Herd B 29%.

Within years there was a correlation of .42 between weaning grade and weaning weight. The square of this correlation is .18, thus 18% of the variance in weaning grades within years was due to variation in weaning weight. Sex, herd and pasture differences in weaning grade were adequately explained on the basis of this correlation.

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THE EFFECT OF PASTURE CROPS WITH AND WITHOUT SUPPLEMENTAL CORN FEEDING AND DRY-LOT MANAGEMENT FOR BEEF PRODUCTION AND NEMATODE CONTROL

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DIET and dietary supplements have been shown to exert a profound influence upon the resistance of a host to disease. Experimental tests of a single food were probably begun when Zimmerman *et al.* (1926) reported that adequate vitamin B complex increased the resistance of a host to the nematode, *Ascaridia galli*. Vitamin A was shown to have a similar influence (Ackert *et al.*, 1927; 1931). Vitamin D protected the host from the effects of ascarid parasitism rather than retarding parasite development (Ackert and Spindler, 1929).

With respect to protein supplements Ackert and Beach (1933) reported that chickens fed meat scraps and skim milk in addition to a basal cereal ration harbored fewer and shorter ascarids than chickens receiving the same basal ration supplemented with meat scraps or peanut meal. Riedel and Ackert (1950, 1951) noted that a supplement of 14.2% soybean oil meal and skim milk every second day produced greater resistance among birds than did either a supplement of 14.2% soybean oil meal or 14.2% meat scraps in a similar basal ration.

A generally deficient diet may be expected to reduce the resistance of dogs to the incidence of hookworms (Foster and Cort, 1931); and the same condition has been reported for rats with respect to the tapeworm, *Hymenolepis fraterna* (Shorb, 1933). Riedel (1949) presented data to show that mice harbored fewer *Trichinella spiralis* when they were fed a balanced diet contaning whole milk. Taylor (1943) infected three groups of lambs with equal numbers of trichostrongylid larvae and fed three different rations. At autopsy he found that the lambs fed a full ration of hay with concentrates harbored 3,000 worms, those fed hay alone had 9,000 nematodes, while the remaining group fed straw alone had 14,000 worms per animal.

Vegors *et al.* (1955) reported that calves grazed on pasture had more

[1] The authors of this report are indebted to Dr. Walter J. Drapala, Statistician, Mississippi Experiment Station, State College, Mississippi, for analysis of data presented in this research.

worms than those fed corn as a supplement to the same pasture crop, and calves grazed on fescue harbored more worms than those grazed on either temporary winter ryegrass, oats or crimson clover. They believed that the poor nutritional quality of fescue was in part the cause of a higher incidence of parasitism. Data presented by Riedel (1955) indicated that the nematode larval mortality on fescue pastures was much lower than it was on ryegrass pasture, and this difference may eventually result in a higher degree of parasitism in cattle grazed on fescue.

The object of this study was to note the nematode potential and weight gain of steers as affected by dry-lot management, and pasture crops with and without supplemental corn feeding.

Experimental Procedure

The animals used in this research were mixed breeds of Angus, Herefords, and Shorthorns selected so that the breed in one group was represented in each of the other experimental groups. The term of grazing of each group expired when the grazing became inadequate or the crop matured.

At the beginning and every 28 days thereafter each of the steers was weighed. Fecal samples were collected from the cattle on weigh days. From the individual fecal samples the nematode ovum content was determined qualitatively by the zinc flotation method and quantitatively by the Stoll procedure. After the grazing season the nematode larval incidence was determined on the forage of each pasture by the procedure previously used by Riedel (1955).

The data were collected in 1953 and 1954. The weight records of the cattle, the numbers of animals shedding parasite ova, the numbers of nematode ova passed by each host and the parasite larval count on the vegetation of each pasture (except in dry lot) served as the criteria.

Results and Discussion

The data in table 1 show the growth records of the groups of steers fed the various pasture crops and the diets in dry-lot. The daily growth records achieved by the groups of animals used during the first year of experimentation correlated closely in most cases with the daily gains of the steers fed similar diets during the second year of investigation. Statistical analysis showed that the significantly higher growth performance of the cattle in dry-lot the second year over those of the first year may have resulted from a higher nutritive quality of alfalfa hay than that of the lespedeza hay used the previous year, a higher parasite

TABLE 1. WEIGHT RECORDS AND PARASITE DATA OF THE GROUPS OF STEERS

Diet group and year	No. of steers	Days on test	Average weight (lb.)			Average nematode egg count[a] (gm. of feces)		No. of steers passing nematode ova	
			Initial	Final	Daily gain	Initial	Final	Initial	Final
1953 Corn-CSM (7:1) ad lib. and 3 lb. lespedeza hay	5	126	563	832	2.13	46	no count	5	2
1954 Corn-CSM (7:1) ad lib. and 3 lb. alfalfa hay	6	75	529	735	2.75	16	6	4	2
Average			545	779	2.36	31			
1953 Fescue-ladino	5	97	601	725	1.28	33	59	3	4
1953 Fescue-ladino	5	97	592	732	1.44	39	19	4	3
1954 Fescue-ladino	8	75	524	624	1.33	20	46	5	6
Average			564	682	1.35	30	41		
1953 Fescue-ladino and ½ rat. cracked corn[b]	5	97	578	775	2.03	6	6	2	4
1954 Fescue-ladino and ½ rat. cracked corn[b]	8	75	526	679	2.01	12	4	5	5
Average			546	716	2.04	9	5		
1953 Ryegrass	5	119	561	784	1.87	26	19	4	3
1953 Ryegrass	5	119	545	780	1.97	6	13	2	2
1954 Ryegrass	8	75	496	684	2.51	24	13	6	5
Average			564	682	2.14	18	15		
1953 Ryegrass and ½ rat. cracked corn[b]	5	119	529	787	2.17	19	13	4	3
1954 Ryegrass and ½ rat. cracked corn[b]	8	75	496	684	2.51	66	12	7	5
Average			278	724	2.37	42	12		
1954 Wheat pasture	8	70	527	702	2.50	132	no count	5	1

[a] Ovum and larval data indicated the following parasites in each group of steers: *Haemonchus, Oesophagostomum, Trichostrongylus, Ostertagia, Cooperia,* and *Chabertia.*

[b] A half ration of corn was half the amount consumed by the steers fed a full ration of corn.

load of the hosts used the first year, or a combined effect of both. A statistically significant growth difference was also evident between the second-year ryegrass group of steers and the two groups of animals used the first year, but this difference may have been due to a variation in the quality of the pasture crops.

An analysis of variance indicated that the average daily weight gains of the steers grazed on fescue-ladino pasture were significantly lower than the average daily weight gains of any of the remaining groups of steers used in the experiment. A highly significant weight increase resulted when the fescue-ladino grazing was supplemented with a half ration of corn (table 1); nevertheless, the weight increase remained significantly below the daily weight gains of the animals grazed on wheat pasture, a full ration (ad libitum) of corn and cottonseed meal (7:1) supplemented with 3.0 lb. of alfalfa hay per animal in dry-lot, an excellent stand of ryegrass pasture (1954), or a ryegrass pasture supplemented with a half ration of corn.

Ryegrass pasture supplemented with a half ration of corn indicated an increased trend of growth over the animals grazed on a ryegrass pasture, but the increase was not significant.

Wheat pasture promoted excellent growth performance. Examination of the data in table 1 showed that the average daily weight gains of the steers grazed on wheat pasture were as high or higher than the weight gains of animals fed any of the other pasture crops with or without a corn supplement. Furthermore, wheat pasture was found to be as satisfactory for promoting weight gains as a dry-lot diet of corn and cottonseed meal (7:1), ad libitum, supplemented with alfalfa hay.

The total parasite ovum count per animal indicated that the animals grazed on a fescue-ladino pasture showed no reduction of the number of parasite ova shed into the pasture during the term of grazing. The reduction of parasite ovum count when corn supplemented fescue-ladino pasture was not significant.

A ryegrass pasture did not affect a significant reduction of the total average number of nematode ova shed into the pasture during the interim of grazing, but the reduction became pronounced when corn supplemented ryegrass.

Wheat pasture and a dry-lot feed of 3.0 lb. of legume hay with free-choice corn and cottonseed meal (7:1) were about equally effective for the reduction of parasite ova shed by the hosts. Wheat pasture was significantly better than any of the other pasture crops (table 1).

The numbers of steers passing nematode ova among the diet groups

were compared. It was found that wheat pasture, although the observations were limited to one year, was as favorable as dry-lot management with a full corn and cottonseed meal (7:1) ration supplemented with legume hay for reducing the number of hosts passing ova. The remaining pasture crops with or without a partial corn supplement did not promote a reduction of hosts shedding parasite ova during the grazing period.

A series of parasite larval determinations was made from vegetation collected from each of the pastures during the final year of experimentation. The data showed a contamination of 164 larvae per 8-lb. sample of vegetation from the fescue-ladino pasture. The number of larvae recovered from the fescue-ladino pasture grazed by animals whose diet was supplmented with corn was 56 larvae for a similar quantity of vegetation, but this reduction correlated with the smaller number of parasite ova shed into the pasture by the grazing hosts. The number of infective nematode larvae recovered from 8-lb. samples of vegetation taken from each of the remaining pastures, much lower than those from the fescue-ladino groups with or without corn, were as follows: ryegrass 14, ryegrass and corn supplement 21, and wheat 2. The soil of the dry-lot area was not sampled for the presence of parasite larvae.

The nematode larvae recovered from the fescue-ladino pasture and a similar pasture grazed by animals whose diet was supplemented with corn belonged in order of frequency to the following genera: *Ostertagia, Oesophagostomum, Haemonchus, Trichostrongylus, Cooperia,* and others not classified. Among the animals whose diet contained ryegrass, the stomach worm, *Haemonchus contortus,* was more prevalent than the nodular worm, *Oesophagostomum* spp. Otherwise, the order of frequency was similar to that of the larvae from the fescue-ladino pastures. The number of larvae obtained from the wheat pasture was too few to establish a generic order of frequency.

Summary

A fescue-ladino pasture yielded a lower beef production than any of the pasture crops and dry-lot rations tested; and as interpreted from the reduction of the average nematode egg counts during the term of grazing, a decrease of the number of hosts shedding nematode ova, and the nematode larval count of vegetation, the nematode parasite potential was the highest in steers grazed on this type of pasture.

A partial corn supplement fed in addition to fescue-ladino grazing significantly increased beef production, but the parasite potential remained unchanged. The same quantity of corn supplementing ryegrass

grazing did not significantly augment beef production from ryegrass, but it did lower the parasite potential.

Wheat pasture was as effective for beef production as a full-feed of corn and cottonseed meal (7:1) supplemented with 3.0 lb. of legume hay in dry-lot, or ryegrass pasture supplemented with corn.

Wheat pasture and the dry-lot diets were equally superior to any of the diets tested for the reduction of the nematode parasite potential of the hosts.

The parasite potential was determined from species of *Ostertagia, Oesphagostomum, Haemonchus, Trichostrongylus, Cooperia, Chabertia, Strongyloides, Bunostomum* and *Nematodirus.*

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THE ESTIMATION OF THE TOTAL DIGESTIBLE NUTRIENTS IN ALFALFA FROM ITS LIGNIN AND CRUDE FIBER CONTENT

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An estimation of the nutritive value of roughages from a simple chemical analysis would materially add to the precision of formulating rations for ruminants. Lignin has been used successfully by some workers to estimate energy or organic matter digestibility, (Lancaster, 1943; Phillips and Loughlin, 1949; Forbes and Garrigus, 1950a, 1950b; Homb, 1952; Richards and Reid, 1953; and Walker and Hepburn, 1955). The histological study by Drapula *et al.* (1947) indicated that most fecal particles were lignified and it appeared that only lignified particles were in the feces. Heller and Wall (1940) and Crampton and Jackson (1944), however, were not able to correlate lignin content with digestibility. The lack of agreement may be due to the mixture and species of the roughages studied. For example, Phillips and Loughlin (1949) obtained far different regression equations for timothy and alfalfa and emphasized that correlation of digestible energy and lignin content were very satisfactory within the species but not between species. In addition Pigden (1953) showed histologically that the progress and site of lignification were not similar in the grasses he studied. It seems probable therefore, that the most satisfactory regression of the nutritive value of a roughage on lignin content would be with one plant species or group of plant species which have a similar site and progress of lignification.

High correlations have been obtained between crude fiber of roughages and digestibility of the organic matter (Axelsson, 1949, 1950; McMeekan, 1943; Lancaster, 1948; Hallsworth, 1949; Walker and Hepburn, 1955). The regression equations obtained by the above workers were very similar. Crampton (1940), Crampton and Jackson (1944) and Thomas and Ibbotson (1947), however, reported that little correlation could be obtained between crude fiber and digestibility of roughages. It has also been reported that even if correlations could be obtained between crude fiber and digestibility, the results were not as satisfactory as the use of lignin, (Phillips and Loughlin, 1949; Forbes

and Garrigus, 1950a; Homb, 1952). A great variety of roughages and mixtures of roughages were used and the lack of agreement of the above workers may be due to variation in recovery rates of lignin in the crude fiber. Armstrong *et al.* (1950) have reported, for example, that 67 to 70% of the lignin is recovered in the crude fiber of alfalfa and trefoil, while only 13 to 28% of the lignin was recovered from the crude fiber of rye, orchard and fescue grass. In general, it might be concluded that crude fiber has not been as satisfactory as lignin when correlated with digestibility.

The work reported herein involves 152 digestion trials on 31 alfalfa hays of great variation in quality in order to establish regression equations which would be useful in predicting the total digestible nutrient (TDN) content from lignin or crude fiber analyses.

Experimental

Wether sheep were used in latin square designed digestion trials conducted over a period of three years. The alfalfa hay was fed at 105 to 125% of their maintenance requirements. A constant daily level of hay was fed during the 7-day preliminary and 7-day collection period. Data were not used from animals refusing to clean up their daily ration at any time during the trial.

The lignin was determined by the method of Ellis *et al.* (1946), while crude fiber and nitrogen were determined by the standard AOAC procedures (1945). Total digestible nutrients were calculated by the method described by Lofgreen (1953). Statistical procedures were those described by Snedecor (1946).

Results and Discussion

Results of the digestion trials (table 1) showed a great range in alfalfa quality (36.2 to 63.4% TDN). The inverse relationship between lignin content and TDN is expressed graphically in figure 1. The regression equation of TDN on lignin content was:

$$Y=84.57-3.21X$$

where Y is percent TDN on a dry basis and X is percent lignin on a dry basis. Both the regression coefficient ($b=3.21$) and the correlation coefficient ($r=-0.88$) were statistically highly significant. The standard deviation from regression was 2.71% and was as accurate as the standard deviation generally found in most of our digestion trials.

The use of crude fiber content to estimate TDN (figure 2) was almost as reliable as lignin content. Here:

$$Y=79.7-0.84X$$

where Y is percent TDN and X is percent crude fiber. The correlation coefficient was —0.86 and the standard error of the estimate was 2.96%. Since Armstrong *et al.* (1950) demonstrated that 67 to 70% of lignin was recovered in crude fiber of alfalfa, it might be expected that the

TABLE 1. ANALYSIS OF ALFALFA HAYS (DRY BASIS)

Hay	No. digestion trials	Lignin	Crude fiber	Nitrogen	TDN
		%	%	%	%
1	4	13.43	47.3	1.65	36.2
2	4	13.03	43.8	1.79	47.5
3	4	12.56	43.0	1.70	42.7
4	4	12.19	39.0	2.47	43.0
5	2	10.94	33.9	2.92	54.2
6	8	10.64	32.2	2.64	50.5
7	2	10.50	33.8	2.79	55.5
8	4	10.41	27.4	2.80	50.9
9	4	10.38	32.0	2.52	48.1
10	4	10.35	35.4	3.19	54.9
11	4	10.20	31.7	2.93	54.9
12	8	10.17	36.5	2.76	51.6
13	8	10.05	34.6	2.78	52.3
14	4	9.77	30.1	2.53	55.0
15	4	9.74	29.0	2.59	53.7
16	4	9.67	31.4	3.14	51.4
17	4	9.58	25.9	2.78	55.4
18	8	9.32	33.7	2.60	52.3
19	4	9.29	....	2.55	54.4
20	4	9.18	30.3	3.05	58.6
21	4	9.13	27.1	2.36	57.0
22	4	8.95	26.9	3.04	58.0
23	8	8.48	30.3	2.89	53.2
24	8	8.41	26.4	3.24	54.2
25	4	8.41	25.0	3.28	59.3
26	8	8.32	26.5	3.62	56.8
27	4	8.20	26.6	3.10	59.3
28	4	7.90	26.2	3.20	60.7
29	4	7.74	26.5	3.32	59.1
30	4	7.39	23.2	3.34	63.4
31	8	7.27	25.4	3.03	58.4

crude fiber of alfalfa might be almost as accurate as lignin in regression equations predicting the TDN of alfalfa. Phillips and Loughlin (1949). however, found in their work that crude fiber was not as closely correlated as lignin to dry matter digestibility.

Correlation of nitrogen content with TDN was 0.77. The regression equation (figure 3) had a standard error of the estimate of 3.68%

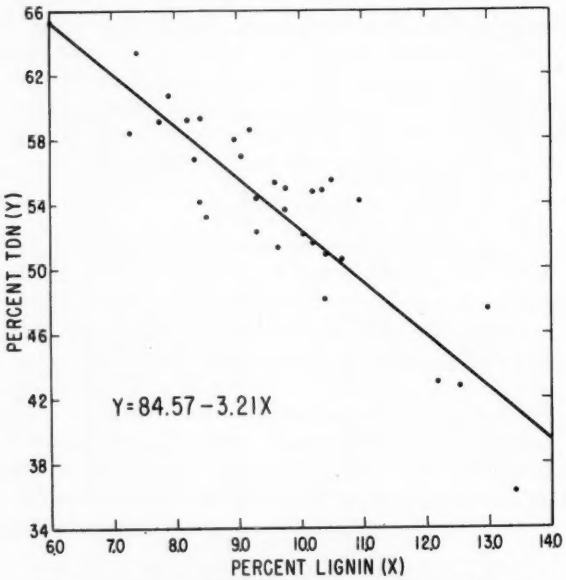

Figure 1. The relationship of lignin content and total digestible nutrients (TDN).

which was not as satisfactory as that of either crude fiber or lignin.

When organic matter digestibility was related to lignin, crude fiber, or nitrogen content, the correlation coefficients were practically identical to those relating TDN to lignin, crude fiber or nitrogen. This was to be expected since Lofgreen (1953) was able to show a very close relationship between organic matter digestibility and TDN.

A multiple regression of TDN on lignin and nitrogen did not prove to be any more satisfactory than a simple regression of TDN on lignin. The standard deviation from regression in this case was 2.75%.

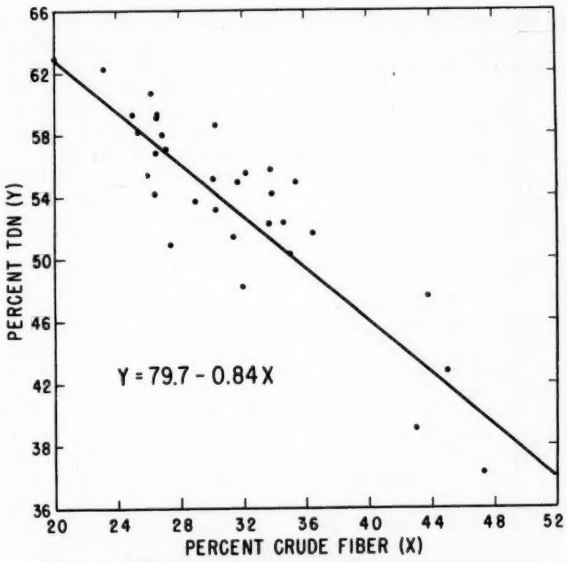

Figure 2. The relationship of crude fiber content and total digestible nutrients (TDN).

As a check on the reliability of the regression equations developed in this work, the data of Forbes and Garrigus (1950b) were used. Only the conventional digestion trial data on alfalfa from which TDN could be calculated was compared to the predicted TDN by our regression equations. Table 2 presents these results. The lignin regression equation was the most satisfactory followed closely by crude fiber. Nitrogen was the least reliable.

TABLE 2. COMPARISON OF CONVENTIONALLY DETERMINED TDN AND PREDICTED TDN [a]

	Predicted from		
Conventional	Lignin	Crude fiber	Nitrogen
65.4	63.4	61.5	60.9
63.0	62.1	61.4	59.2
65.0	65.0	64.7	60.8
59.8	60.8	56.0	58.4

[a] The data of Forbes and Garrigus (1950b) were used for this table.

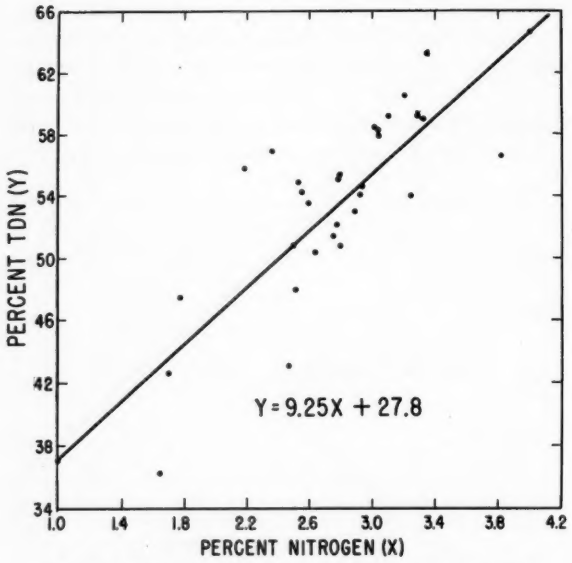

Figure 3. The relationship of nitrogen content and total digestible nutrients (TDN).

It should be emphasized that these equations should be applied only to alfalfa and not to other forages or mixtures of forages unless it is shown that lignification proceeds as it does in alfalfa.

Summary

Experiments were conducted with 152 digestion trials on 31 samples of alfalfa hay of varying quality to relate the total digestible nutrient (TDN) content to either the lignin, crude fiber, or nitrogen content. Regression equations were developed which allow one to predict with reasonable accuracy the TDN content from a lignin or crude fiber analysis. The nitrogen content was shown to be related to the TDN; however, nitrogen was not as closely related as was crude fiber or lignin.

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THE SYNTHESIS OF VITAMIN B_{12} AFTER ORAL AND PARENTERAL ADMINISTRATION OF INORGANIC COBALT TO COBALT-DEFICIENT SHEEP

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IT is now generally agreed that the oral administration of cobalt will prevent or cure cobalt deficiency in ruminants. The experimental evidence indicates that the rumen bacteria incorporate orally administered cobalt into the vitamin B_{12} molecule, an essential metabolite of the animal tissues (Tosic and Mitchell, 1948).

However, there is some disagreement as to the effect of parenterally administered cobalt. The investigators who reported that the injection of cobalt will bring about a slow recovery hypothesized that this recovery is due to the transfer of cobalt from the blood stream or saliva to the rumen where vitamin B_{12} is synthesized (Keener and Percival, 1950; Ray *et al.*, 1948). Monroe *et al.* (1952) reported that there was a higher tissue concentration of radio-active "B_{12}-like" material after intravenous injection of Co^{60} than after oral administration to normal sheep. Although microbiological assays were not run, paper chromatography of the "vitamin B_{12}-like" extracts from several representative samples showed a behavior similar to a solution of pure vitamin B_{12} labeled with Co^{60}. These investigators hypothesized that the administration of cobalt parenterally is ineffective in curing cobalt deficiency as reported by Marston and Lee (1949) and Smith *et al.* (1950) because the vitamin B_{12} synthesized in the tissue from injected cobalt is physiologically ineffective.

The purpose of this experiment was to test the hypothesis of the tissue synthesis of vitamin B_{12}, using a microbiological assay, and to measure the extent of synthesis in the gastro-intestinal tract of cobalt-deficient sheep following oral, intravenous, and subcutaneous administration of cobalt.

Experimental Procedure

The 15 ewe lambs used in this study averaged 65 lb. in weight and were selected from a carload lot of Whiteface western feeders. After

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parasite treatment, the lambs were group-fed a low cobalt ration consisting of shelled yellow corn, powdered skim milk (Borden) and grass hay of low cobalt content (0.04 p.p.m.). The lambs were gradually brought on feed until they were consuming an average of 450 gm. of corn, 100 gm. of skim milk powder, and all the chopped hay they would eat. On this ration they gained as much as 0.20 lb. per day, and reached an average weight of 80 lb. before deficiency symptoms became evident in about 6 months.

The lambs were then moved to individual pens to permit accurate measurement of feed intake. They were offered a ration of 180 gm. corn, 40 gm. skim milk powder, and 500 gm. of hay. None of the lambs consumed this ration completely, and as the deficiency progressed feed intake, especially of concentrates, continued to decrease. The lambs were given access to plain salt blocks and were offered water once daily. Body weights and blood samples for hemoglobin and vitamin B_{12} analyses were taken every 28 days for the first 7 months. Thereafter they were taken at 2-week intervals.

The lambs were continued on the deficient ration until they showed definite signs of cobalt deficiency—anorexia, loss in weight, and anemia (approximately 7 month). They were then divided into treatment groups. Only 14 of the original sheep were available for treatment. Since the sheep did not develop cobalt deficiency at a uniform rate it was not possible to start treatment of all the sheep at one time.

The treatments consisted of (1) cobalt, orally for 1 and 2 weeks; (2) cobalt, intravenously for 1 and 2 weeks; (3) cobalt, subcutaneously for 2 weeks; and (4) negative controls. All animals that received cobalt were treated daily with 1 mg. of cobalt as $CoCl_2 \cdot 6H_2O$. Two lambs were allotted at random to each treatment. The negative control group consisted of four animals. One lamb that received cobalt subcutaneously died the third day after treatment.

At the end of the treatment period blood samples were taken for B_{12} analysis and the animals were sacrificed. The liver, kidneys, spleen, adrenals, and pancreas were removed, weighed, frozen and kept at 20° F. along with the blood samples until they could be assayed for vitamin B_{12}. The gastro-intestinal tract was removed, and the contents of the following segments were taken: rumen-reticulum, omasum-abomasum, duodenum-jejunum, ileum, cecum, and large intestine. The contents of each section were removed quantitatively and frozen at 20° F. until they could be assayed for vitamin B_{12}. Rosenthal *et al.* (1952) reported that serum samples could be stored in the frozen state

(—20° C.) for 6 months without any perceptible loss of B_{12} activity. In this laboratory, gastro-intestinal contents were found to vary from +15 to —30% in B_{12} activity after storage at 20° F. for a period of 11 months.

All samples except the adrenal were assayed at least twice for vitamin B_{12} with *lactobacillus leichmannii* ATCC 4797 by the method of Peeler *et al* (1949). Five-gram samples of the frozen tissues were homogenized with 50 ml. of acetate buffer, pH 5.0, in a micro Waring blendor. One mg. of NaCN was added to each sample, and they were then autoclaved at 15 lb. pressure for 30 minutes. The samples were made up to 100 ml. volume and filtered under suction. The filtrate was diluted for assay according to its B_{12} content.

The gastro-intestinal samples were allowed to thaw before 5-gm. samples were taken. Fifty ml. of acetate buffer, pH 5.0, and 1 mg. NaCN were aded to each sample. They were autoclaved for 30 minutes at 15 lb. pressure, and then brought up to 100 ml. volume before filtering. The filtrate was then diluted for assay according to its B_{12} content.

Twenty-five ml. of acetate buffer, pH 5.0, and 1 mg. of NaCN was added to 5 ml. of each blood sample. After autoclaving for 30 minutes at 15 lb. pressure, the samples were brought up to 50 ml. volume and filtered under suction. This filtrate was added directly to the assay tubes in measured amounts.

Crystalline vitamin B_{12} added to the samples was recovered to the extent of 108, 96 and 90% in the gastro-intestinal samples, tissue samples, and blood samples, respectively. This is well within the experimental error of microbiological assays. The response of the organism after 16 hours incubation at 39° C. was measured turbidimetrically with an Evelyn Photoelectric colorimeter at 660 mμ.

The moisture of the gastro-intestinal contents was determined by the toluene method. The tissue samples were weighed frozen and dried in the oven at 100° C. for dry matter determination.

Results

Table 1 present the vitamin B_{12} concentration in the blood, and body weight changes of 15 lambs on the preliminary cobalt-deficient ration. The lambs gained in weight for the first 18 weeks on the deficient ration; however, the vitamin B_{12} level in the blood after an initial rise declined. No reason can be given for the rise in B_{12} in the blood the first 4 weeks on the deficient ration. The zero- and four-week

TABLE 1. VITAMIN B_{12} CONCENTRATION IN THE BLOOD, AND BODY WEIGHTS OF LAMBS ON A COBALT-DEFICIENCY RATION

(15 lambs)

Weeks on deficient ration	Vitamin B_{12} in the blood	Body weight
	mmcg. per ml.	lb.
0	0.55 (0.04)[a]	65 (0.7)[a]
4	1.11 (0.03)	69 (1.1)
8	0.30 (0.02)	72 (1.6)
12	0.22 (0.02)	72 (2.0)
16	0.08 (0.01)	78 (2.5)
18	0.10 (0.01)	80 (3.1)
20	0.09 (0.01)	77 (3.5)
24	0.15 (0.04)	73 (4.2)

[a] Mean and standard error.

samples were assayed at the same time, and should be comparable. All 15 animals had higher levels at 4 weeks than at the start of the experiment. Variance was small. It would seem that the reduced level of vitamin B_{12} in the blood can be used as a specific symptom of cobalt deficiency in ruminants.

Table 2 shows the vitamin B_{12} concentration in the blood of the cobalt-deficient lambs immediately prior to treatment and at slaughter time. As can be seen from the preliminary values all the animals had a low level of vitamin B_{12} in the blood. After treatment only the lambs that received cobalt orally showed a significant increase in vitamin B_{12} in the blood. The level of B_{12} in the blood of lambs that received any other treatments remained relatively constant.

Table 3 presents the vitamin B_{12} concentration of body tissues and of the contents of the gastro-intestinal tract of cobalt-deficient lambs

TABLE 2. VITAMIN B_{12} CONCENTRATION IN THE BLOOD OF COBALT-DEFICIENT SHEEP GIVEN DIFFERENT TREATMENTS

Treatment	Pretreatment values	Slaughter values
	mmcg. per ml.	mmcg. per ml.
Cobalt-deficient ration (4)	0.10 (0.05)[a]	0.11 (0.05)
Cobalt orally (4)	0.10 (0.01)	0.62 (0.07)**
Cobalt I. V. (4)	0.07 (0.04)	0.09 (0.05)
Cobalt Subcut. (1)	0.10	0.12

[a] Mean and standard error.
( ) No. of animals.
** Difference significant at the 1% level.

after treatment. The size of some of the standard errors indicates the large variation observed.

All treatment means were compared to the negative control means, using the t test. $t = \frac{d}{\sqrt{\frac{1}{n_1}+\frac{1}{n_2}(\hat{E})}}$, where $\hat{E}$ was calculated from the analysis of variance.

TABLE 3. THE VITAMIN B_{12} CONCENTRATION OF BODY TISSUES AND CONTENTS OF THE GASTRO-INTESTINAL TRACT OF TREATED COBALT DEFICIENT LAMBS

(Values in mcg. per 100 gm. dry matter)

Section	Cobalt deficient ration (4)	Cobalt orally (4)	Cobalt, 1 mg. per day	
			I. V. (4)	Subcut. (1)
		1 mg. per day		
Liver	7 (2.6)[a]	26 (7.3)	8 (2.9)	5
Kidney	46 (5.6)	84* (5.2)	47 (6.6)	28
Spleen	12 (3.8)	26 (6.6)	15 (4.0)	15
Pancreas	3 (0.9)	12* (2.8)	5 (1.9)	0
Adrenal	5 (0.7)	11* (1.7)	5 (0.9)	5
Rumen-reticulum	15 (2.7)	104* (24.0)	21 (5.3)	46
Omasum-abomasum	8 (2.2)	78* (25.3)	20 (4.4)	24
Duodenum-jejunum	12 (3.1)	123** (22.3)	17 (6.2)	12
Ileum	17 (5.3)	236** (27.8)	32 (12.5)	31
Cecum	51 (8.6)	157 (31.0)	209 (72.4)	183
Large intestine	55 (4.5)	228 (65.4)	219 (86.0)	276

[a] Mean and standard error.
* Difference significant at the 5% level.
** Difference significant at the 1% level.
( ) No. of animals.

Because there was no apparent difference between groups, the data were combined for the one- and two-week treatment periods for both the cobalt orally and cobalt intravenously injected groups. Compared to the negative controls, the kidneys, pancreas and adrenals of the lambs that received cobalt orally contained a significantly greater concentration of vitamin B_{12}. The other tissues of the treated groups did not differ significantly from the negative controls, although the concentration of vitamin B_{12} in the liver of animals that received cobalt orally approached significance. The contents of the rumen-reticulum, omasum-abomasum, duodenum-jejunum and ileum of the lambs that received cobalt orally contained a significantly higher concentration of vitamin B_{12} than the negative controls. Although the

cecum and large intestine of all treatment groups contained a greater concentration of vitamin B_{12} than the negative controls, they did not approach significance primarily because of the large variance and the small number of animals. Generally speaking the contents of the rumen-reticulum, omasum-abomasum, duodenum-jejunum and ileum of the animals that received cobalt parenterally contained a higher concentration of vitamin B_{12} than the negative controls. Although this was not

TABLE 4. TOTAL VITAMIN B_{12} CONTENT OF THE BODY TISSUE AND CONTENTS OF THE GASTRO-INTESTINAL TRACT OF TREATED COBALT-DEFICIENT LAMBS

(Values in micrograms)

Section	Cobalt deficient ration (4)	Cobalt orally (4)	Cobalt, 1 mg. per day	
			I. V. (4)	Subcut. (1)
		1 mg. per day		
Liver	5[a] (1.5)	38** (7.6)	7 (2.5)	3
Kidney	8 (1.2)	15* (1.0)	7 (1.0)	4
Spleen	1 (0.3)	2 (0.5)	2 (0.5)	1
Pancreas	0.1 (0.0)	0.6* (0.2)	0.2 (0.1)	0
Adrenal	0	0.1	0.1	0
Rumen-reticulum	53 (14.3)	480** (92.7)	85 (14.4)	70
Omasum-abomasum	5 (1.5)	63* (21.8)	8 (2.7)	29
Duodenum-jejunum	1 (0.3)	29* (9.9)	3 (1.1)	1
Ileum	3 (1.1)	63* (18.3)	6 (2.2)	2
Cecum	50 (4.7)	190* (28.9)	157* (31.5)	172
Large intestine	21 (3.9)	98* (20.0)	84* (6.5)	61

[a] Mean and standard error.
* Difference significant at the 5% level.
** Difference significant at the 1% level.
( ) No. of animals.

significant, it suggests that some of the injected cobalt found its way into the upper alimentary tract.

The total vitamin B_{12} content of the body tissues and the gastro-intestinal contents of the cobalt-deficient lambs after treatment is shown in table 4.

Compared to the negative controls, the liver, kidney, and pancreas of lambs fed cobalt contained significantly greater quantities of vitamin B_{12}. All other tissues of treated lambs did not differ significantly from the negative controls. The contents of all of the gastro-intestinal segments of the lambs that received cobalt orally contained significantly more vitamin B_{12} than the negative controls. The cecum and large intestine of the lambs that were injected with cobalt intravenously con-

tained significantly more vitamin B_{12} than the controls. Although the quantity of vitamin B_{12} present in most of the other segments of the treated animals was higher than in the negative controls, it was not significant.

In this study the tissues listed in decreasing order of concentration of vitamin B_{12} after oral administration of cobalt were as follows: kidney, liver, spleen, pancreas, and adrenal. In the negative controls and in those given cobalt parenterally, the tissues in order of decreasing concentration of the vitamin were as follows: kidney, spleen, liver, adrenal, and pancreas.

Yacowitz *et al.* (1952) reported the concentration of vitamin B_{12} in the tissue of chicks on a B_{12}-supplemented diet to be as follows: kidney, liver, pancreas, adrenals, and spleen in decreasing order of concentration. The chick and cobalt-supplemented sheep store vitamin B_{12} similarly in the tissues, other than the spleen.

The largest quantity of vitamin B_{12} in the tissues of the sheep that received cobalt orally was in the liver, the kidney, spleen, pancreas, and adrenals following in decreasing order of total vitamin B_{12} present. Comar and Davis (1947) reported similar depositions of labeled cobalt in the tissues of a 30-day-old calf after the oral administration of 60 mcg. In cobalt-deficient lambs the kidney contained the largest quantity of vitamin B_{12} followed by the liver, spleen, pancreas, and adrenals.

The administration of cobalt orally was the only treatment which significantly increased the amount or concentration of vitamin B_{12} in the body tissues. This was presumably due to the absorption of vitamin B_{12} from the alimentary canal following its synthesis by the rumen microflora. The concentration and total amount of vitamin B_{12} in the omasum-abomasum was less than in the rumen-reticulum for all treatment groups, indicating that vitamin B_{12} disappeared from the omasum-abomasum probably by absorption.

Whether or not vitamin B_{12} was absorbed from the small intestine could not directly or indirectly be determined. Vitamin B_{12} was apparently synthesized in the lower intestinal tract as indicated by the increased concentration of vitamin B_{12} as well as an increase in the total amount present in all treatment groups. The significant increase of vitamin B_{12} in the cecum and large intestine of the lambs that received cobalt parenterally is evidence of lower intestinal synthesis of vitamin B_{12} from cobalt that is known to be excreted via the bile into the small intestine. Evidently this vitamin B_{12}, synthesized in the

lower intestinal tract, is relatively unavailable as shown by the low tissue levels of vitamin B_{12} in the lambs that received cobalt parenterally.

This study failed to demonstrate the synthesis of vitamin B_{12} by the body tissues as reported by Monroe *et al.* (1952). Evidently the increased "vitamin B_{12}-like" fraction which they observed after the injection of cobalt is physiologically inactive for *Lactobacillus leichmannii* as well as for sheep.

Thus is seems that the slow response of cobalt-deficient lambs to parenterally administered cobalt (Keener and Percival, 1950; Ray *et al.*, 1948) is best explained by the transfer of cobalt from the blood stream or saliva to the rumen which permits limited synthesis of vitamin B_{12}. The data presented here support this hypothesis, since the total amount and the concentration of vitamin B_{12} was greater, though not significantly so, in the rumen-reticulum and omasum-abomasum of the lambs that received cobalt parenterally than in the negative controls. With the administration of larger doses of cobalt for a longer period of time, the synthesis of vitamin B_{12} in the rumen might be sufficient to elicit a physiological response.

Summary

The level of vitamin B_{12} in the blood of sheep on a cobalt-deficient ration decreased rapidly. However, the lambs continued to eat well and gained weight for some time after the level of B_{12} in the blood declined to low levels. This suggests that tissue vitamin B_{12} is efficiently conserved.

The administration of cobalt orally to cobalt-deficient lambs significantly increased the vitamin B_{12} level in the blood, kidney, pancreas, adrenals, rumen-reticulum, omasum-abomasum, duodenum-jejunum and ileum; the total vitamin B_{12} content was significantly increased in the liver, kidney, pancreas, rumen-reticulum, omasum-abomasum, duodenum-jejunum, ileum, cecum and large intestine.

The parenteral administration of cobalt to the deficient lambs failed to significantly increase the vitamin B_{12} level in the blood or body tissues. However, the vitamin B_{12} content in the cecum and large intestine was significantly increased, presumably as a result of bacterial synthesis following the excretion of cobalt into the duodenum via the bile.

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THE SULFUR REQUIREMENT OF GROWING-FATTENING LAMBS IN TERMS OF METHIONINE, SODIUM SULFATE, AND ELEMENTAL SULFUR [1, 2]

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UREA as a partial nitrogen replacement for protein nitrogen is becoming more and more extensively used in ruminant rations.

Loosli and Harris (1945) showed that the addition of methionine to rations containing urea increased nitrogen rentention by lambs. Hale and Garrigus (1953), Block *et al.* (1951), and Block and Stekol (1950) through the use of labeled inorganic sulfur (S^{35}), showed that the ruminant can utilize and convert inorganic sulfur into wool and milk proteins. Starks *et al.* (1953), using the paired feeding technique, showed that elemental sulfur could be used by lambs to supply partially their dietary needs for sulfur when added to a ration containing urea and formulated to be low in sulfur. Thomas *et al.* (1951) showed that the addition of sulfate salts to sulfur deficient diets increased daily gains of lambs.

Lofgreen *et al.* (1953) were unable to show any improvement in lamb gains from the addition of inorganic sulfate to a urea-containing ration which contained 0.2% total sulfur before supplementation. Starks *et al.* (1954) compared the performance of lambs fed varied levels of elemental sulfur, sodium sulfate, and methionine, in a purified ration containing 4% urea. Albert *et al.* (1955) found the addition of 0.2% elemental sulfur to certain modified urea supplements in silage wintering rations containing 0.12% total sulfur improved lamb gains. Whiting *et al.* (1953) studied the sulfur requirements of mature ewes and stated that their data indicated the sulfur requirement of a mature ewe does not exceed approximately 0.1% of the total ration.

The experiment reported here was designed as a further comparative

[1] This is the seventh paper in a series on sulfur in sheep nutrition.

[2] Grateful acknowledgement is extended to the E. I. duPont deNemours Company, Inc., Wilmington, Deleware, for kindly providing the urea; to Merck and Company, Rahway, New Jersey, courtesy of Dr. H. H. Draper, for generously supplying thiamine hydrochloride and riboflavin; to Vio Bin Corporation, Monticello, Illinois, courtesy of Steve Varner, for generously supplying the wheat germ oil; to Dow Chemical Company, Midland, Michigan, courtesy of Dr. R. W. Colby, for kindly furnishing methionine; and to Calcium Carbonate Company, Quincy, Illinois, courtesy of W. A. Hensley, for generously supplying the mineral mixture used in this study.

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study of the sulfur requirements of growing-fattening lambs fed a purified ration with urea as the major nitrogen source.

Experimental Procedure

Growth studies employing individual feeding and a purified ration in which urea furnished 92% of the nitrogen were used to compare the quantitative requirements of growing-fattening lambs for methionine, sodium sulfate, and elemental sulfur.

Forty-five grade black-faced lambs of similar breeding, with initial weights of 36 to 66 lb., varying 38 days in age, produced at the Illinois Dixon Springs Station were used in this study. Outcome groups of five lambs of nearly equal weight were formed. Each of three groups was divided randomly among five levels of one of the three sulfur sources. Because of three deaths and one refusal to eat the ration, the data reported here are based upon 41 lambs. One of these 41, receiving 0.12% methionine, died two days before the end of the experiment, having lost 11 lb. while consuming 76 lb. of feed. These data have been used as if they came from the full experimental period.

During the 56-day trial, beginning June 1, 1953, and terminating July 27, 1953, each lamb was confined to a 4-by-4-foot pen bedded with wood shavings. The partitions separating the pens were covered with half-inch-mesh wire. Each lamb was allowed to consume its ration ad libitum and had access to water at all times. Individual feed records were kept. The barn and pens were sprayed periodically to control flies. Each lamb was weighed at the beginning of the test and at two-week intervals, between 9 and 10 a.m.

The basal purified ration (table 1), to which the sulfur sources were added, is the one used by Starks *et al.* (1954). Total nitrogen determinations according to the Kjeldahl-Wilfarth-Gunning method (A.O.A.C., 1950), were made on all rations. All rations were analyzed for total sulfur according to the A.O.A.C. method (1950). The supplement levels, the analyzed sulfur, the total nitrogen of the basal ration and the N:S ratios are given in table 2.

The lowest level of sodium sulfate was intended to be approximately equivalent in total sulfur to that of the lowest level of elemental sulfur. Succeeding levels of these two sources were also intended to be approximately equal in total sulfur. Each level of methionine was intended to contain approximately one-fourth of the amount of supplemental sulfur in the corresponding level of elemental sulfur and sodium sulfate, the work of Starks *et al.* (1954) having indicated that such levels of

TABLE 1. COMPOSITION OF PURIFIED RATION

Ingredient	Percent
Corn starch	35.4
Cerelose	22.5
Wheat straw	15.0
Wood flock	15.0
Corn oil	3.5
Wheat germ oil	0.5
Urea (Crystalline)	4.0
Choline chloride	0.1
Minerals	4.0[a]
Vitamins A, D, B_1, B_2	+ [b]

[a] A complete mineral mixture.
[b] Viadex guaranteed to contain 4,000 U.S.P. units of vitamin A and 750 U.S.P. units of vitamin D per gram at rate of one gram per pound of ration. Thiamine and riboflavin at rate of one milligram each per pound of ration.

methionine would probably include the optimum. Lamb gains served as the chief basis of evaluating sulfur requirements.

At the beginning of the test period duplicate wool samples were clipped by the use of a small-animal Oster clipper from a measured area on the right fore rib of each lamb. The wool samples taken at the

TABLE 2. COMPOSITION OF RATIONS

Ration [a]	Analyzed sulfur	Ratio N:S	
		Analyzed [b]	Calculated [c]
%	%		
Basal	0.048	42.1	
Basal+0.10 S	0.109	18.5	13.6
Basal+0.25 S	0.252	8.0	6.8
Basal+0.40 S	0.412	4.9	4.5
Basal+0.55 S	0.560	3.6	3.4
Basal+0.70 S	0.711	2.8	2.7
Basal+0.44 T	0.111	18.2	13.7
Basal+1.10 T	0.278	7.3	6.8
Basal+1.76 T	0.416	4.9	4.5
Basal+2.42 T	0.571	3.5	3.4
Basal+3.08 T	0.722	2.8	2.9
Basal+0.12 M	0.057	35.4	27.3
Basal+0.29 M	0.088	23.0	18.4
Basal+0.47 M	0.115	17.6	13.6
Basal+0.64 M	0.153	13.2	10.9
Basal+0.82 M	0.202	10.0	9.0

[a] S, elemental sulfur; T, sodium sulfate; M, DL-methionine.
[b] Basal analyzed 2.02 N.
[c] Using basal sulfur by analysis plus added sulfur.

beginning of the study were used to adjust the weight of the wool grown during the experimental period. After taking the original wool samples all the wool was clipped from approximately a five-square-inch area on the right fore rib. After 54 days on the test ration, the wool from each lamb was again clipped from approximately 50 square centimeters of this five-square-inch area. Individual wool samples were washed, dried and weighed by the method used by Starks *et al.* (1953).

Results and Discussion

Table 3 summarizes the data on rate of gain and feed intake for each of the sulfur sources. The three lambs receiving the lowest level of elemental sulfur (0.10%) lost weight during the test. One lamb gained

TABLE 3. EFFECTS OF SULFUR LEVELS UPON RATE OF GAIN AND FEED EFFICIENCY OF LAMBS

	Elemental sulfur				
Number of lambs	3	3	3	3	3
Levels, percent of ration	0.10	0.25	0.40	0.55	0.70
Average daily gain per lamb, lb.	−0.071	0.208	0.286	0.226	0.185
Average feed, lb. per lamb per day	1.22	2.07	2.31	1.93	1.80
	Sodium sulfate				
Number of lambs	3	3	2	3	3
Levels, percent of ration	0.44	1.10	1.76	2.42	3.08
Average daily gain per lamb, lb.	0.196	0.226	0.223	−0.036	0.054
Average feed, lb. per lamb per day	2.15	2.33	2.50	1.15	1.73
	Methionine				
Number of lambs	3	2	2	2	3
Levels, percent of ration	0.12	0.29	0.47	0.64	0.82
Average daily gain per lamb, lb.	−0.190	0.098	0.357	0.321	0.315
Average feed, lb. per lamb per day	0.95	1.87	2.88	2.27	2.21

3 lb.; the other two lost 5 and 10 lb., respectively. Weir *et al.* (1952) reported that lambs fed hay containing 0.08 to 0.12% total sulfur grew more slowly than lambs fed hay with 0.22% total sulfur.

The lambs receiving the lowest level of methionine (0.12%), with a total sulfur content of 0.057% analyzed sulfur, lost weight during the study. As mentioned above, one lamb on this lowest level of methionine died two days before the termination of the study having lost 11 lb. The other two lambs lost 10 and 11 lb., respectively. Starks *et al.* (1954) found that lambs fed a similar ration containing 0.058% total sulfur lost weight or died during a 70-day trial.

The lambs receiving the two highest levels of elemental sulfur, 0.55 and 0.70%, made smaller daily gains than the lambs receiving 0.4% elemental sulfur. Those receiving 0.70% made the smallest daily gain.

Starks *et al.* (1954) also found a decline in gains at 0.6% elemental sulfur.

The lambs used in 1953 receiving the two highest levels of sodium sulfate, 2.42 and 3.08%, made smaller daily gains than those receiving 1.10 or 1.76%. Christensen *et al.* (1947), testing sulfur in lamb fattening rations to prevent overeating, found that large amounts of sulfur in the diet reduced feed intake. There is no obvious explanation of the fact that the lambs receiving the 2.42% sodium sulfate ration ate less feed and gained at a slower rate (and even lost weight) than lambs receiving a higher level, 3.08% sodium sulfate. Dick (1953) reported that high levels of dietary sulfate interfere with molybdenum and copper metabolism.

The lambs receiving the rations containing the three highest levels of methionine all made good gains although those on the highest level, (0.82%), gained less than those on the next two lower levels. As the lambs receiving the lowest level of methionine showed negative gains, the data suggest that 0.12% methionine probably did not furnish sufficient sulfur to permit good growth.

The levels of 0.4% elemental sulfur, 1.10% sodium sulfate, and 0.47% methionine permitted the largest gains, respectively, for the three sulfur sources. Starks *et al.* (1954) found that the lambs fed a similar ration supplemented with 0.40% elemental sulfur, with 1.31% sodium sulfate, or with 0.5% methionine permitted the largest gains.

Covariance analysis of average daily gains showed that initial weight was useless as a statistical control for gains, the regression coefficient being only —0.00028 lb. per day per pound of initial weight, agreeing with experience that initial weight is useful only when the gain is relatively large, say as large as the average intial weight, and showing that nothing was accomplished by the use of outcome groups. Analysis of variance of gains, ignoring outcome groups, found highly significant differences between gains of lambs on different levels for each of the sulfur sources. The gain data for elemental sulfur and methionine could be fitted (by least squares) by a quadratic curve, and it would reasonably be expected that the sodium sulfate data could also. However, this was not the case. It appears that some accident affected the gains at the 2.42% sodium sulfate level, and it was felt that this level should be omitted in estimating the sodium sulfate level that would permit maximal lamb gains. The analysis of variance is given in table 4, the inclusion of all three sulfur sources being justified by reasonable equality of their respective error mean squares.

From the fitted quadratic curves, the levels of the various sulfur sources that permitted the largest daily gains were estimated to be 0.47±0.028 elemental sulfur, 1.27±0.44 sodium sulfate, and 0.64± 0.048 methionine %, respectively. The respective sulfur contents are 0.47±0.028, 0.29±0.099, and 0.138±0.010.

Approximately 70% less sulfur was needed as methionine in the diet fed than as elemental sulfur, or about 50% less than as sulfate sulfur, for optimum performance. The sulfur requirement for sodium sulfate in terms of total sulfur appears somewhat smaller than that

TABLE 4. ANALYSIS OF VARIANCE OF AVERAGE DAILY GAINS

Source of variation	Degrees of freedom	Mean square	Variance ratio	Probability
Sulfur sources	2	.0067	(1.18)	n.s.
Quadratic curves:				
Elemental sulfur	2	.1071	13.6	0.001—
Sodium sulfate	2	.0287	3.64	0.05—
Methionine	2	.2838	36.0	0.001—
Pooled deviations	5	.0051	(1.55)	n.s.
Omitted point	1	.1001	12.7	0.01—
Pooled error	26	.00789		

of elemental sulfur. This tends to support the idea of Starks *et al.* (1953) that sodium sulfate is more efficiently used than elemental sulfur.

As the lambs were fed individually, feed consumption data were available and were used to seek further understanding of the observed response to the various sulfur sources. Covariance analysis of feed consumed and average daily gains found a significant relation, as would be expected, but adjustment to equal feed consumption did not obliterate the differences originally found. The adjusted differences were significant at the 5% level for elemental sulfur, and at the 1% level for methionine, and for each the contribution of a quadratic regression coefficient was significant at the 5% level. Thus there is a clear indication that the effect of varying the level of these sulfur sources is not merely an effect on feed intake.

It is of interest that, for sodium sulfate, the adjusted differences are not significant, suggesting that the accident which we presume affected the average daily gain observed on 2.42% sodium sulfate was that the three animals on that level were an uncommon sample as regards their willingness to eat the diet offered, though no such conclusion can

be justified. While feed consumption was significantly affected by sulfur level in the diet, and while this suggests that palatability may be a factor in the response measured as average daily gain, the reader should not lose sight of the chief objective of this experiment, which was to ascertain the optimum level of sulfur for ad libitum feeding of the three sources tried.

Loosli (1952) suggested that the sulfur requirement of sheep might be inferred roughly from tissue analyses. He theorized from tissue analyses that one part of sulfur is needed for each 15 parts of nitrogen in the diet. The calculated nitrogen to sulfur ratio (table 2) of the 0.47% methionine level is 13.6:1, which is close to Loosli's suggested ratio. A larger methionine sulfur requirement, such as the calculated optimum 0.64 ± 0.048, would narrow this to 10.9:1. The calculated ratio for 0.40% elemental sulfur level was 4.5:1 and for 1.10% sodium sulfate was 6.8:1. If one assumes 50% digestibility for elemental sulfur, as suggested by Starks *et al.* (1954), and the same figure for sodium sulfate these ratios then would become 9:1 for elemental sulfur and 13.6:1 for sodium sulfate.

It will be noted in table 2 that analysis of rations containing added sulfur did not recover the total sulfur presumably present as judged by analysis of the basal ration plus the amount added, allowing some doubt as to the N:S ratios. Callan and Toennies (1941) reported a loss of 19–22% methionine sulfur through the use of the Parr-bomb oxidation method. Bert (1955) states that his findings showed approximately 15% loss of methionine sulfur using this method. However, a percentage loss will not account for the irregularities in our data, average recovery of added sulfur exceeding 99%.

Hartsook and Mitchell (1954) found that age greatly influenced the methionine sulfur requirement of rats. Their data showed a decline in sulfur requirement with advancing age. In practice there may be considerable variability in ages of lambs fed for slaughter. During this study the lambs changed in age from about 4 to about 7 months. It may be that the sufur requirements of older lambs or for mature sheep are somewhat lower.

Statistical analysis of the wool yields showed that neither elemental sulfur nor sodium sulfate had a significant effect upon wool growth. The addition of methionine to the purified ration significantly increased wool growth. The data indicated that wool yields were increased approximately 0.02 gm. per square centimeter for each percent of added methionine. Starks *et al.* (1954) found that sulfur-supplemented lambs

grew significantly more wool than lambs receiving a basal ration analyzing 0.054% sulfur. While Starks *et al.* (1954) reported that neither the sulfur sources nor the sulfur levels used had any significant effect upon wool growth, Garrigus *et al.* (1950) found that the addition of one-half percent methionine to a basal ration low in sulfur-bearing amino acids significantly increased wool growth of lambs.

Analysis of Combined Data

Two tests, 1952 and 1953, with reference to the sulfur requirements of growing-fattening lambs, have been conducted at the Illinois Agricultural Experiment Station. Since the age and breeding of the lambs,

TABLE 5. LEVELS OF SULFUR SOURCES USED IN 1952 AND 1953 TESTS

Elemental sulfur		Sodium sulfate		Methionine	
1952	1953	1952	1953	1952	1953
%	%	%	%	%	%
....	0.10	0.89	0.44	...	0.12
0.20	0.25	1.33	1.10	0.2	0.29
0.40	0.40	1.78	1.76	0.5	0.47
0.60	0.55	....	2.42	0.7	0.64
....	0.70	....	3.08	...	0.82

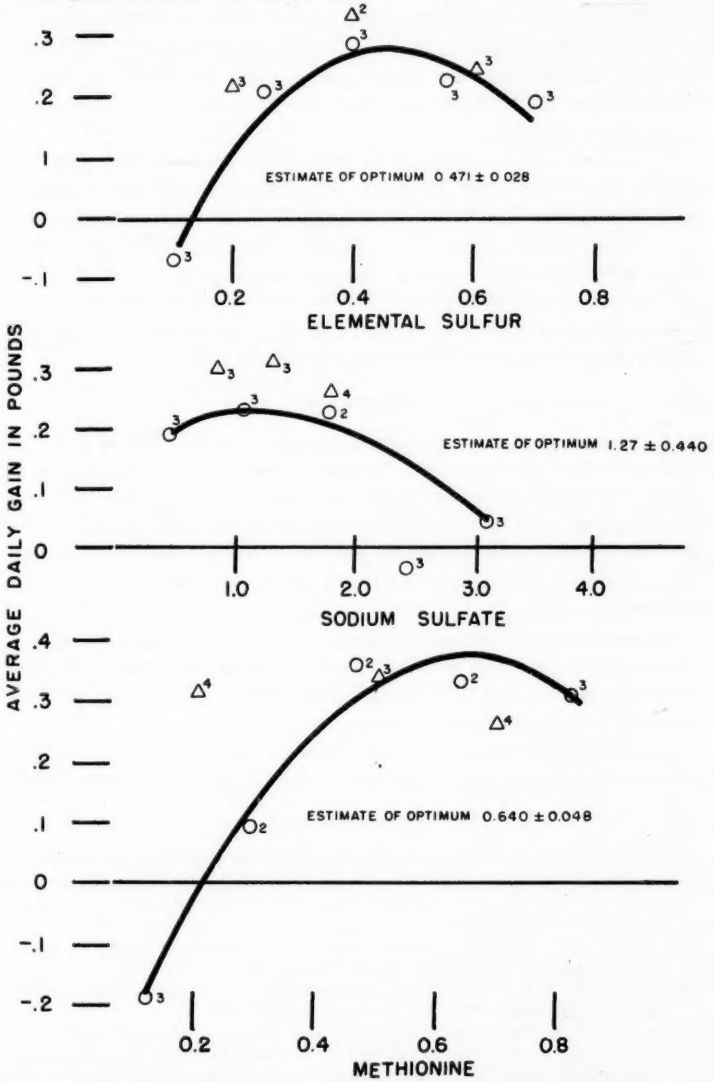
the sulfur sources compared, and the basal rations used in the two studies were similar, a combined analysis is reported here for weight gains as affected by sulfur sources and sulfur levels. The 1952 data were reported by Starks *et al.* (1954). The 1953 data are those presented in this paper. In the first test the lambs were fed during a period of 70 days, while the second test lasted only 56 days. The percentage levels of various sulfur sources added to the basal rations are shown in table 5.

Figure 1 shows the weight gains of lambs as affected by the level of elemental sulfur, sodium sulfate, or methionine, respectively, in the diet. The curves have been fitted to the 1953 data only.

The lambs of the two tests that received comparable levels of elemental sulfur made similar gains and the gains followed similar curves. In each test the lambs receiving a ration with 0.4% added elemental sulfur made the largest average daily gains. This close agreement in gains at this level supports the estimated optimum level of 0.47± 0.028% found from the 1953 data.

In comparing performances as affected by level of sodium sulfate

Figure 1. Relation of Gain to percent sulfur source in the ration.*



* The small numerals indicate the number of lambs represented by each point. △ 1952 data. ○ 1953 data.

(disregarding the fourth level, 2.42%, of the second study), the pattern of gains is similar in the two studies. In 1952, the lambs receiving 1.33% of sodium sulfate made the largest gains; in 1953, the lambs receiving 1.10% made the largest gains. These two levels of sodium sulfate agree well with the estimated optimum level of $1.27 \pm 0.44\%$ based on the 1953 data.

Comparing gains as affected by methionine level, in both tests the largest gains were made on rations supplemented with approximately 0.5% methionine. The data show that the 1952 lambs made good gains on 0.2% methionine, but the 1953 lambs receiving 0.29% methionine made only fair gains. The fact that gains declined in both tests when the rations contained 0.64 and 0.70% methionine agrees reasonably with the estimated optimum $0.64 \pm 0.048\%$ in suggesting that the optimum level lies somewhere in the vicinity of 0.64%.

Summary

A comparative study was made of the sulfur requirements of growing-fattening lambs in terms of methionine, sodium sulfate, and elemental sulfur, added to a purified ration containing 4% urea. The gains on five levels of each of the sulfur sources fitted a quadratic curve. On the basis of the fitted curves, the calculated sulfur percentage requirement was for methionine 0.64 ± 0.048, for sodium sulfate 1.27 ± 0.44, and for elemental sulfur 0.47 ± 0.028, respectively.

On the basis of total sulfur about 70% less sulfur was needed as methionine in the diet than as elemental sulfur, or about 50% less as sulfate sulfur. The data suggest that sodium sulfate may be used more efficiently than elemental sulfur.

Satisfactory agreement was found between the sulfur requirements of growing-fattening lambs as reported in studies of two different years.

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UREA AND STILBESTROL FOR FATTENING LAMBS[1,2]

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PROFITS from lamb feeding depend, to a large extent, on efficient feed utilization and rapid gains. Added profits could be realized by greater use of low-quality, lower-cost feeds. Several areas of the United States produce an abundance of low-protein roughages. Were it possible to effectively supplement these roughages with low-cost non-protein nitrogen compounds, lower feed costs would allow for smaller necessary margins in lamb-feeding operations. The use of urea and related compounds in supplementing low-protein feeds has been studied extensively. Results with lambs have not been consistent. The earlier research has been summarized in an excellent review by Reid (1953).

Iowa workers (Rath Packing Company and Iowa State College cooperating) (1953) reported results of lambs fattened on a protein supplement of soybean oil meal, linseed oil meal, and urea. The urea in the supplement provided approximately 10% of the protein equivalent of the ration. Gains of the lambs on the blended supplement was equal to the gains of lambs receiving linseed oil meal as the supplement. The gains of lambs fed the urea supplement were more economical than those of lambs fed the linseed oil meal. Richard and Dinusson (1954) obtained good results when urea supplied 12% of the protein equivalent of the ration of fattening lambs.

The use of hormones and hormone-like compounds has been shown to stimulate rates of gain and feed efficiency. Stilbestrol, implanted subcutaneously, has significantly increased rate of gain and feed efficiency. Andrews *et al.* (1949a; 1949b), Jordan (1950), Pope *et al.* (1950), Klosterman *et al.* (1951), Bell *et al.* (1954), and Richard and Dinusson (1954) all reported that rates of growth were increased and that more efficient gains were produced by stilbestrol implants. Although beneficial in these respects, some investigators have reported undesirable side effects from subcutaneous implantation of stilbestrol pellets in fattening lambs. Andrews *et al.* (1949a), Pope *et al.* (1950), and Bell

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[2] Published with permission of the Director.

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et al. (1954) reported lower carcass grades for lambs so treated. Jordon (1953) reported large losses in commercial feed lots from prolapse of the rectum and vagina. He further reported losses from uremia in treated wether lambs. Bell *et al.* (1954) described similar symptoms in treated wether lambs.

Experimental Procedure

Seventy-two uniform grade Columbia feeder lambs that averaged 66 lb. in weight were selected for this experiment. These lambs had been drenched with phenothiazine and given free access to prairie hay prior to the start of the experiment. After a suitable rest period they were allotted into eight groups at random. All lots were fed native Junegrass hay ad libitum. This hay contained 5.7% crude protein by analysis. The grain mixtures used during this trial are shown in table 1.

TABLE 1. FORMULAS OF GRAIN MIXTURES

Ingredients	Control ration	Urea ration
	lb.	lb.
Corn, yellow No. 2	1574	1574
Soybean oil meal (44%)	400	....
Supplement U[a]	....	400
Steamed bonemeal	20	20
Sodium sulfate	6	6
	2000	2000

[a] Supplement U was 85% corn and 15% urea.

The stilbestrol treatments were superimposed, giving a 2×4 factorial design. Stilbestrol was mixed with the concentrate to give daily intakes shown in table 2. All lots were hand fed twice daily as much of the mixture as they would readily consume in 20 minutes. All lambs were given free access to water.

Results and Discussion

All groups of lambs had good appeties and "stayed on" feed throughout the feeding period. The lambs that received stilbestrol were more active and also more aggressive at the feed bunk. The stilbestrol intakes, average daily feed intakes, and the average daily gains of the lambs are presented in table 2.

It is of interest to note the roughage to concentrate ratio. These lambs were fed native Junegrass hay ad libitum. Grain was hand fed

TABLE 2. AVERAGE DAILY GAINS AND FEED INTAKES, POUNDS

	0.0 mg. Stilbestrol	0.5 mg. Stilbestrol	1.0 mg. Stilbestrol	2.0 mg. Stilbestrol
	Soybean oil meal supplement			
Lot No.	I	VII	III	V
Grain	2.59	2.89	2.76	2.65
Hay	1.15	1.18	1.14	1.13
Gain	0.50	0.53	0.53	0.59
	Urea supplement			
Lot No.	VIII	II	VI	IV
Grain	2.25	2.40	2.35	2.54
Hay	1.18	1.12	1.12	1.17
Gain	0.33	0.45	0.46	0.50

to what the lambs would readily consume in 20 minutes. The lambs fed stilbestrol tended to increase the concentrate intake but not the hay. The grain intake is much greater in relation to hay than has usually been experienced at this station. Presumably the low hay consumption was due to the low quality of the hay fed during the experiment.

The lambs fed the soybean oil meal-supplemented ration consumed, on the average, 12% more grain and gained 20% faster than those fed the urea supplement. There was no difference in amounts of hay eaten. These results are not in line with work reported earlier from this station, Richard and Dinusson (1954). In this experiment, urea made up approximately 41% of the protein equivalent of the ration as compared to 23% in the previous experiments. This higher level of urea may account for the poorer performance of these rations in this experiment.

A summary of the 10-week mean gains and analysis of variance of pertinent data are presented in tables 3 and 4. It is seen that the lambs receiving the soybean oil meal supplement made greater gains than the

TABLE 3. SUMMARY OF THE 10-WEEK MEAN GAINS

Stilbestrol level	Soybean oil meal supplement	Urea supplement	Means [a]
mg.	lb.	lb.	lb.
0.0	34.8	25.2	30.0
0.5	37.0	31.7	34.3
1.0	40.3	31.9	36.4
2.0	41.1	35.1	38.1
Means, lb.[a]	38.5	31.0	....

[a] Least significant difference was 4.2 lb. for level means at 5%.

TABLE 4. ANALYSIS OF VARIANCE OF 10-WEEK GAINS

Source	d.f.	s.s.	m.s.	f
Protein source	1	1007.25	1007.25	25.1**
Stilbestrol level	3	654.76	218.30	5.4**
Protein × stilbestrol	3	62.96	20.99	0.5
Error	61	2449.76	40.16	
Total	68	4174.73		

** Significant at 0.01 level.

lambs on the urea ration. These differences were highly significant. All levels of stilbestrol gave significant increases over none and with each increase in level there was a further increase in gain. The response was greatest between no stilbestrol and the 0.5 mg. level and the smallest increment of increase was between the 1.0 and 2.0 mg. levels. The variation resulting from interaction of sources of protein with levels of stilbestrol was not significant, indicating no relationship between type of protein and stilbestrol. Although not indicated in the table, the response to stilbestrol by sex of lambs was non-significant. The wether lambs were slightly heavier than the ewe lambs at the beginning of the experiment and consequently gained slightly faster, but the differences in rate of gain between sexes was not large enough to be significant.

Carcass Evaluation

At the conclusion of the feeding period all lambs were sent to the local packing plant where they were held overnight and slaughtered. Data were collected on cold carcass weights. Each lamb was graded by a competent grader and numerical values were assigned to each market grade: choice-plus equaled 10, choice-average equaled 9, etc. A summary of the carcass data is given in table 5.

TABLE 5. AVERAGE CARCASS GRADES AND DRESSING PERCENTAGES

Stilbestrol level	Carcass grades		Dressing percentages	
	Soybean oil meal supplement	Urea supplement	Soybean oil meal supplement	Urea supplement
mg.				
0.0	7.4	8.2	48.4	50.0
0.5	8.0	7.7	48.8	47.7
1.0	8.3	8.0	48.0	47.7
2.0	8.0	7.5	48.0	47.4

There were no significant differences in carcass grades or yields. Most groups graded low choice to high good. These grades are considered average for Columbia-type lambs of similar weights.

Summary

Results from a factorial experiment involving 72 Columbia-type lambs on two different protein supplements and four levels of stilbestrol are presented. Soybean oil meal was a significantly better protein supplement than urea when urea (42% N) was fed at the rate of three % of the concentrate mixture with poor quality non-legume hay (urea furnished 41% of the nitrogen in the ration). Feeding stilbestrol produced significantly greater gains on both types of nitrogen. There were no significant differences in carcass grades or yields as a result of stilbestrol treatment. No death loss nor observable deleterious effects were attributable to the stilbestrol.

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THE EFFECT OF ORAL AND SUBCUTANEOUS ESTROGEN AND ANDROGEN ADMINISTRATION ON GROWTH AND CARCASS QUALITY OF LAMBS[1]

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DURING the past decade it has been shown that growth rate, efficiency of feed utilization, and carcass composition and grade can be altered by hormone administration. In both wether and ewe lambs the subcutaneous implantation of compressed diethylstilbestrol, dienestrol, or estradiol and progesterone pellets has consistently improved rate of gain and increased feed efficiency (Andrews *et al.*, 1949; Jordan, 1950; Pope *et al.*, 1950; O'Mary *et al.*, 1952; Galloway *et al.*, 1952; Means *et al.*, 1953; Clegg *et al.*, 1954, 1955; Bell *et al.*, 1954; Whiting *et al.*, 1954; Wilkinson *et al.*, 1955.) In general the above studies have shown a reduction in carcass quality as measured by official Federal carcass grades when diethylstilbestrol or dienestrol have been administered. The principal factors contributing to the reduction in carcass grade appear to be a reduction in internal and external fat deposition, and an increase in the moisture content of the external fat (O'Mary *et al.*, 1952; Clegg and Cole, 1954; Wilkinson *et al.*, 1955.)

The purpose of this study was to compare estrogens and androgens, singly and in combination, orally and subcutaneously, in an attempt to produce the beneficial effects on growth and feed efficiency without the undesirable effects on carcass quality.

Materials and Methods

Sixteen lots of 12 Western feeder lambs each were used. The lambs averaged 79 lb. at the beginning of the trial on November 19, 1954 and were fattened for a period of 73 days until January 31, 1955. Each treatment was duplicated; thus, there were 24 lambs assigned to each treatment. Both ewe and wether lambs were used, and most lots contained equal numbers of each.

All lambs received a ration of shelled corn, soybean oil meal, alfalfa hay and iodized salt. The treatments of each lamb were as follows: Lots 1 and 2, controls; Lots 3 and 4, one 12-mg. pellet of diethylstil-

[1] Contribution from the Department of Animal Husbandry, Journal Paper No. 911, Purdue University, Agricultural Experiment Station, Lafayette, Indiana.

bestrol implanted subcutaneously at the base of the ear; Lots 5 and 6, eight pellets containing a total of 10 mg. of estradiol and 250 mg. of progesterone implanted subcutaneously beneath the lower jaw; Lots 7 and 8, 2 mg. of dienestrol per lamb daily, orally; Lots 9 and 10, 4 mg. of dienestrol per lamb daily, orally; Lots 11 and 12, 25 mg. of methyl testosterone per lamb daily, orally; Lots 13 and 14, 12.5 mg. of methyl testosterone and 2 mg. of dienestrol per lamb daily, orally; Lots 15 and 16, 25 mg. of methyl testosterone and 2 mg. of dienestrol per lamb daily, orally. The hormones which were administered orally were dissolved in corn oil and added to soybean oil meal in amounts which would provide the daily oral hormone dose in 0.2 lb. of soybean oil meal. The hormones were fed until the close of the experiment on January 31. On that day each lamb was individually weighed in duplicate.

The lambs were shipped a distance of 65 miles by truck and were slaughtered on February 1, 1955. The lambs were distributed at random in the cooler and the carcasses were graded according to U. S. Federal standards by a government grader. A complete half-carcass was selected at random from each treatment for estrogen assay. The carcass was boned and the muscle and fat were ground and mixed in a food chopper. Samples were taken for chemical analysis and the remainder of the meat was air-dried and incorporated in a standard mouse all-grain ration in a ratio of five parts by weight of dried meat to one part of the grain ration. Four sheep livers were obtained from each treatment. The liver was ground, air-dried, and reground in a Waring blendor. The dried liver was assayed without incorporation in the grain ration of the mice. The meat and liver were assayed by oral administration to castrate female mice of the Rockland-Swiss strain for 10 days. The meat-grain and dried liver were fed at a rate of 3 gm. per mouse per day. The mice were sacrificed 24 hours after the last feeding and the uteri removed and weighed. Details of the assay procedure are described by Stob *et al.* (1954).

Results

Growth Rate

The data on total gains and daily gains per lamb are summarized in table 1. An analysis of variance of total gain showed that the subcutaneous implantation of 10 mg. of estradiol and 250 mg. of progesterone produced a highly significant increase in gain ($P<0.01$) and that both the subcutaneous implantation of 12 mg. of diethylstilbestrol

TABLE 1.THE EFFECT OF HORMONES ON GROWTH RATE, FEED CONSUMPTION AND FEED UTILIZATION OF LAMBS
(November 19, 1954 to January 31, 1955. 73 days)

	I	II	III	IV	V	VI	VII	VIII
Treatments	Control	DES pellets	Estradiol-progesterone pellets	2 mg. Dienestrol orally	4 mg. Dienestrol orally	25 mg. M. Testosterone orally	2 mg. Dienestrol 12.5 mg. M. Test. orally	2 mg. Dienestrol 25 mg. M. Test. orally
No. per treatment	22	23	23	23	22	24	22	23
				Growth rate				
Av. initial wt., lb.	74.6	75.1	74.4	74.3	74.4	74.9	74.8	74.1
Av. final wt., lb.	101.1	110.5	111.3	103.4	107.4	102.1	104.9	105.8
Total gain, lb.	26.5	35.4*	36.9**	29.1	33.0*	27.2	30.1	31.7
Av. daily gain, lb.	.37	.49	.51	.40	.45	.37	.41	.43
				Daily feed consumption				
Shelled corn, lb.	1.17	1.16	1.16	1.14	1.16	1.16	1.18	1.16
Soybean oil meal, lb.	.20	.20	.20	.20	.20	.20	.20	.20
Hay, lb.	2.25	2.30	2.28	2.16	2.30	2.34	2.37	2.26
Minerals, lb.	.01	.02	.02	.02	.02	.01	.01	.01
				Feed per pound gain				
Shelled corn, lb.	3.17	2.41	2.28	2.86	2.58	3.11	2.87	2.73
Soybean oil meal, lb.	.54	.41	.39	.51	.45	.54	.49	.47
Hay, lb.	6.11	4.76	4.46	5.43	5.12	6.28	5.77	5.31
Minerals, lb.	.03	.04	.04	.04	.03	.03	.03	.03
Total	9.85	7.62**	7.17**	8.84*	8.18**	9.96	9.16	8.54**

* $P<0.05$, ** $P<0.01$.

and the oral administration of 4 mg. of dienestrol daily significantly increased total gain ($P<0.05$). The oral administration of 2 mg. of dienestrol per lamb daily did not increase rate of gain significantly.

Feed Consumption and Efficiency

The amount of soybean oil meal per lamb was held constant at 0.20 lb. per lamb daily throughout the experiment. Shelled corn was fed in amounts which were readily cleaned up, and, as shown in table 1, daily corn intake did not vary greatly between treatments. Hay was fed *ad libitum*. The differences in corn required per unit of gain between the control lambs and lots 2, 3 and 5 were 24%, 28% and 22% less than the controls, respectively. The differences in soybean oil meal, in the same order of comparison, were 25%, 30% and 17%. An analysis of variance of total feed required per unit of gain showed that the implantation of either diethylstilbestrol, or estradiol plus progesterone, or the oral administration of 4 mg. of dienestrol daily, significantly increased feed efficiency ($P<0.01$). Although the feeding of 2 mg. of dienestrol daily did not significantly increase rate of gain it appeared to improve feed efficiency ($P<0.05$). The combination of 2 mg. of dienestrol and 25 mg. of methyl testosterone likewise improved feed efficiency ($P<0.01$).

Mammary Stimulation

As in previous studies with suckling or feeder ewe and wether lambs, marked mammary stimulation was produced by estrogen administration. No attempt to quantitate the response in terms of teat length, weight of mammary tissue or composition or volume of secretion were made. During the sixth week of the experiment definite mammary development including both the udder and teats was evident in the ewe and wether lambs which had been implanted with diethylstilbestrol or estradiol plus progesterone. Varying amounts of secretion were obtained from both ewes and wethers. In some instances the secretion was watery or serous in nature and in others resembled milk. In the lambs receiving dienestrol in the feed, at both the 2 and 4 mg. levels, there was definite mammary stimulation and a secretion was obtained from some sheep. It appeared without quantitative evaluation that the degree of stimulation was less than in the estrogen implanted sheep. It is questionable whether there was any stimulation in the testosterone treated lambs. However, there was some teat and mammary develop-

ment in the sheep receiving dienestrol and methyl testosterone in combination.

Carcass Studies

The lambs were individually weighed on January 31, 1955, immediately loaded on trucks, and transported to Indianapolis, a distance of 65 miles.

They were unloaded and weighed in lots by treatments, since it was not possible to obtain individual live weights at the packing plant.

TABLE 2. EFFECT OF HORMONES ON SHRINKAGE AND CARCASS YIELD

Treatment	Av. transit shrink	28-Hr. cooler shrink	Av. dressing	Av. total slaughter loss
	%	%	%	%
Control	1.0	2.9	49.1	51.4
Diethylstilbestrol pellet	1.7	2.9	47.5	53.2*
Estradiol-progesterone pellets	1.4	3.3	47.0	53.5**
2 mg. Dienestrol, orally	1.2	2.6	48.6	51.9
4 mg. Dienestrol, orally	1.6	2.8	47.7	53.1**
25 mg. M. Testosterone, orally	1.0	2.9	49.0	51.5
2 mg. Dienestrol plus 12.5 mg. M. Testosterone, orally	1.4	2.8	46.1	52.4
2 mg. Dienestrol plus 25 mg. M. Testosterone, orally	1.2	2.8	48.7	51.8

* $P<0.05$, ** $P<0.01$.

The difference in lot weight between Purdue and Indianapolis was designated as *transit shrinkage*. The lot weights at Indianapolis and the individual cold carcass weights were used to determine average dressing percentage. These data were not suitable for statistical analysis.

The lambs were slaughtered at 7:30 a.m. on February 1, 1955. Individual warm carcass weights were obtained as the carcasses left the killing floor and individual cold carcass weights were obtained at 1:00 p.m. on February 2, 1955. An analysis was made of the difference in individual live weight at Purdue and cold carcass weight. This difference, expressed in percent, was designated as *total slaughter loss* and should not be confused with the conventional dressing percentage. The lowest transit shrinkage and highest dressing percentage occurred in the control and methyl testosterone groups. Total slaughter loss was greatest in the lambs which received either the estradiol-progesterone

pellets or 4 mg. of dienestrol orally ($P<0.01$). The lambs implanted with a 12 mg. pellet of diethylstilbestrol likewise had a higher slaughter loss ($P<0.05$).

The lamb carcasses were graded by a Federal grader on February 2. The lambs were arranged in the cooler at random and treatments were unknown to the grader. The percentage of carcasses which fell in the conventional grade groups are shown in table 3. Because it is difficult, if not impossible, to express Federal grading standards in numerical terms for statistical analysis, another device was used. A comparison was made of the average value per pound of the carcasses in the various

TABLE 3. EFFECT OF HORMONES ON CARCASS GRADE AND VALUE

Treatment	Carcass grade			Av. value per lb.
	Prime	Choice	Good	
	%	%	%	dollars
Control		95	5	.408
Diethylstilbestrol pellet		52	48	.391
Estradiol-progesterone pellets		62	38	.387*
2 mg. Dienestrol, orally		74	26	.400
4 mg. Dienestrol, orally		45	55	.388*
25 mg. M. Testosterone, orally		83	17	.403
2 mg. Dienestrol plus 12.5 mg. M. Testosterone, orally	5	55	40	.394
2 mg. Dienestrol plus 25 mg. M. Testosterone, orally		78	22	.401

* $P<0.05$.

lots. This was accomplished on the basis of the actual packer selling price per pound of the carcasses at the following rates: Choice, 41 cents; good, 37 cents; and good yearling, 31 cents. These data are summarized in table 3. The administration of estradiol and progesterone pellets, or the feeding of 4 mg. of dienestrol daily did reduce carcass quality ($P<0.05$). The trend in reduction in carcass grade and actual value per pound by the various estrogen treatments has been consistently observed at Purdue and other experiment stations as will be subsequently discussed.

Estrogen Assay

As previously described, one half of a carcass was selected from each treatment, ground, dried and assayed, using the castrate mouse uterine weight technique. The livers from four lambs in each treatment were

TABLE 4. ESTROGEN ASSAY OF LAMB CARCASS

Treatment	No. mice	Av. body wt.	Av. uterine wt.	% body wt.
		gm.	mg.	
Control	9	33.22	18.20	.055
Diethylstilbestrol pellet	10	33.00	19.08	.058
Estradiol-progesterone pellets	10	31.20	22.84*	.073**
2 mg. Dienestrol, orally	10	32.40	21.24	.066*
4 mg. Dienestrol, orally	10	32.50	20.62	.064
2 mg. Dienestrol plus 12.5 mg. M. Testosterone, orally	10	30.30	18.10	.060
2 mg. Dienestrol plus 25 mg. M. Testosterone, orally	10	30.80	17.78	.058

* $P<0.05$, ** $P<0.01$.

composited and assayed in a similar manner. There was a significant increase in the uterine weight of the test mice which received the meat of the lamb treated with the estradiol-progesterone pellets. In terms of diethylstilbestrol, the amount which must be added to an all grain diet to produce a uterine weight of 23 mg. would be less than 0.005 mcg. per gram of ration. The oral administration of dienestrol at levels of 2 or 4 mg. per lamb daily, alone or in combination with methyl testosterone, produced a highly significant increase in the estrogenic activity of the liver. In terms of diethylstilbestrol activity, mouse uterine weights of 28 and 47 mg. would be produced by the consumption of an all grain ration containing 0.01 and 0.02 mcg. of diethylstilbestrol per gram of ration respectively.

The estrogenic activity of the feces was determined at various times

TABLE 5. ESTROGEN ASSAY OF LAMB LIVER

Treatment	No. mice	Av. body wt.	Av. uterine wt.	% body wt.
		gm.	mg.	
Control	10	29.90	18.42	.062
Diethylstilbestrol pellet	10	27.30	17.00	.062
Estradiol-progesterone pellets	10	28.50	18.20	.064
2 mg. Dienestrol, orally	10	27.30	27.64**	.101**
4 mg. Dienestrol, orally	10	28.00	46.96**	.169**
2 mg. Dienestrol plus 12.5 mg. M. Testosterone, orally	10	26.30	24.16*	.092**
2 mg. Dienestrol plus 25 mg. M. Testosterone, orally	10	26.40	27.36**	.103**

* $P<0.05$, ** $P<0.01$.

during the experiment. There was a 5 to 6 fold increase in fecal estrogenic activity in the lambs which received either 2 or 4 mg. of dienestrol orally per day. These data will be presented in a separate publication.

Discussion

As summarized in the introduction there has been almost complete agreement that the subcutaneous administration of diethlystilbestrol, dienestrol, or estradiol plus progesterone in the form of compressed pellets has significantly improved growth rate and feed efficiency in both ewe and wether lambs. As reported by Clegg *et al.* (1955), increased gain following diethylstilbestrol implantation in lambs occurred regardless of age, sex or dietary regime. Much less information is available with respect to the oral administration of hormones or hormone-like substances. Hale *et al.* (1954) found considerable variability in the response to oral diethylstilbestrol. They suggest that there may be a growth response at levels of 2.0 mcg. per pound of ration, a growth depression between 3 and 600 mcg. and an increase in growth above 600 mcg. per pound of ration. The oral administration of dienestrol at considerably greater levels, 4 mg. per lamb per day, appeared to produce a significant growth stimulation in this experiment. Luther *et al.* (1955) observed that the oral administration of 3 mg. of diethylstilbestrol per day, alone or in combination with Terramycin, improved both growth rate and feed efficiency.

The mode of action of hormones in rate of gain and feed efficiency has not been completely explained. Whitehair *et al.* (1953) found that diethylstilbestrol-implanted lambs showed increased nitrogen retention, as did Jordan (1953). Clegg *et al.* (1954) made a similar observation in steers. Clegg and Cole (1954) report that in ruminants diethylstilbestrol administration results in anterior pituitary hypertrophy, increased ACTH secretion and adrenal hypertrophy. They theorize that there is an accompanying increase in androgen production and an acceleration of protein anabolism. By contrast, Whiting *et al.* (1954) concluded that the increased body weight resulting from diethylstilbestrol implantation is due to increased tissue moisture and a greater percentage of offal and that there is no significant difference in feed efficiency.

In a study of the effects of diethylstilbestrol implantation in full-fed and limited-fed lambs, Wilkinson *et al.* (1955) found that diethylstilbestrol increased carcass length, pelt weight and visceral weight and tended to increase muscular development, although the latter increase

was not statistically significant. The evidence available tends to show a true growth stimulating effect.

The data also seem to indicate a systemic action of the hormone. The work of Brooks *et al.* (1954) demonstrating the *in vitro* and *in vivo* effect of stilbestrol on cellulose digestion suggests the possibility of a local action in the rumen. However, it is rather difficult to reconcile a local rumenal action with large oral doses needed to elicit a physiological response. Presumably, the well known difference between oral potency versus intramuscular or subcutaneous effectiveness of a hormone in systemic responses is due to hepatic inactivation of the hormone. If a local rumenal response were involved, then a minute amount of hormone should be effective since the hormone would exert its effect before any inactivation could take place. The data presented in this paper refute the effectiveness of small amounts of hormone orally since the 4 mg. of dienestrol elicited a greater response than 2 mg. For the 73-day feeding period, 4 mg. per lamb daily, a total of 292 mg. orally, was not as effective physiologically as a 12 mg. pellet of stilbestrol subcutaneously. If a systemic phenomenon is involved in the effect of estrogens on growth and feed efficiency then the oral administration of the hormones or hormone-like products is an extremely inefficient method. These differences in efficiency of action depending on route of administration are well known in the laboratory animals.

The effects of estrogens on mammary development, the gonads and various parts of the genital tract are well known for a variety of laboratory animals. Although most experiments involving the use of hormones or hormone-like substances in meat animal production have not included systematic studies of the effects of treatment on mammary or genital function, there is good evidence that these tissues are affected. Perry *et al.* (1951) reported mammary development and secretion in diethylstilbestrol treated ewe and wether lambs, and enlargement and hyperemia of the vulva in ewe lambs. Similar reports of mammary development have been made by Jordan (1953) and Clegg *et al.* (1955). O'Mary *et al.* (1952) found that diethylstilbestrol increased the size of the seminal vesicles and ampulla in wethers and decreased ovarian follicle diameter in ewes. At least two instances of severely unfavorable effects have been reported. Jordan (1953) reported high death losses in two field trials in diethylstilbestrol pelleted lambs. Prolapse of the rectum occurred in wethers and prolapse of the vagina and uterus in ewes, and there was a blockage of the urinary tract. Bell *et al.* (1953) observed a similar response in wethers and on post mortem examination

found enlargement of the Cowpers and prostate glands and partial closure of the urethra.

In meat animal production the evaluation of any experimental procedure must consider rate and economy of gain, safety of treatment to animal and consumer, and economic value of both the live animal and the carcass. The most difficult evaluation thus far has been the effects of hormonal treatment on carcass quality. The chief problem in arriving at a satisfactory analysis of the data arises from the difficulty of assigning numerical values which correctly express the differences in grading standards. It is our opinion that consistent trends in actual field experiments more correctly evaluate the effects of treatment on carcass quality than any statistical device thus far proposed. There is almost complete agreement that the implantation of diethylstilbestrol results in some reduction in carcass grade (Andrews *et al.*, 1949; Jordan, 1950; O'Mary *et al.*, 1952; Clegg *et al.*, 1954; Bell *et al.*, 1954; Luther *et al.*, 1954; Murphree *et al.*, 1954; Wilkinson *et al.*, 1955). Galloway *et al.* (1952) reported that a combination of diethylstilbestrol and progesterone resulted in higher carcass quality than in untreated lambs. O'Mary *et al.* (1952) found that neither testosterone nor progesterone tended to modify the other actions of diethylstilbestrol. Burroughs *et al.* (1954) reported that in steers "the oral administration of diethylstilbestrol produces the desirable effects of pellet implantation without any of the undesirable side effects" and Hale *et al.* (1954) stated that in lambs "diethylstilbestrol administered orally did not appear to have any appreciable effect upon carcass quality."

In the present experiments the best carcasses were from the untreated lambs and the poorest carcasses were from the lambs which received 4 mg. of dienestrol per day orally. Various explantions for the reduction in carcass quality by estrogens have been given. O'Mary *et al.* (1952) explained the reduction on the basis of both lack of fat and an increase in the moisture content of the fat. Clegg and Cole (1954), in describing the effects of diethylstilbestrol on steer, heifer and ewe lamb carcasses, reported less marbling, less internal and external fat deposition, and certain changes in conformation and muscle fiber structure. Recent studies by Wilkinson *et al.* (1955) showed that when estrogen implanted lambs were compared to controls of similar carcass weight and age, the estrogen treated animals had a greater percentage of moisture in the fat, and lower dressing percentage, carcass finish and grade and external fat thickness. "Limited feeding intensified the effect of

estrogen in retarding development of the late-maturing tissues, particularly external fat." By increasing the length of full feeding, carcass grades, external fat covering and moisture content of the fat became similar to the controls. Preliminary Purdue data on chemical composition of the entire edible carcass suggest that lambs which received estrogens either orally or subcutaneously tend to have higher proteins, higher moisture and lower fat content.

In previous work Stob *et al.* (1954) observed that the estrogenic activity of a ration containing meat from animals which received no hormone treatment was greater than that of the all-grain ration used as the stock diet in the mouse colony. The nature of the difference was not fully explored. However, because of the obvious difference in protein of the grain ration (20%) and the grain-meat mixture (35%) casein was added to the grain ration to increase the protein content to 35%. This had no effect on the uterine weight response of the mice. The incorporation of certain legumes in the stock laboratory ration has also been shown to increase its estrogenic activity beyond that of meat and meat products. This emphasizes the widespread distribution of estrogens and some of the problems involved in expressing the estrogenic activity of meat or other substances in quantitative terms. Stob *et al.* (1954) showed that in the case of lambs implanted with 12 mg. of diethylstilbestrol the maximum average uterine weights obtained were about 3 mg. heavier than those produced by meat from non-treated lambs. It is estimated that the estrogenic activity would not exceed 0.1248 mg. per 100 lb. of meat. In some meat samples from lambs implanted with 24 mg. of diethylstilbestrol the maximum estrogenic activity is estimated at 1.135 mg. per 100 lb. of meat. Ellis *et al.* (1954), using an assay procedure involving extraction of tissue and injection of the extract into mice, concluded that the residual estrogen content in 100 gm. of tissue from lambs implanted with 12 mg. of diethylstilbestrol did not exceed 7.1 mcg. in kidney fat, 2.8 mcg. in shoulder muscle and 4 mcg. in liver. In the present research the implantation of 12 mg. of diethylstilbestrol or the oral administration of dienestrol had no significant effect on the estrogenic activity of the meat. Although there appeared to be a significant response from the estradiol-progesterone pellets it represents an extremely minute amount of activity (less than 0.005 mcg. per gram of test ration). The livers of lambs which were fed dienestrol alone or in combination with methyl testosterone did produce a significant increase in mouse uterine weight.

Comparisons with diethylstilbestrol dose response curves indicate that the livers from dienestrol-fed lambs contain estrogens equivalent to 0.01 to 0.02 mcg. of diethylstilbestrol per gram of dried liver.

Summary

The subcutaneous implantation of 10 mg. of estradiol plus 250 mg. of progesterone, or 12 mg. of diethylstilbestrol produced highly significant and significant increases in total gain, respectively. The oral administration of 4 mg. of dienestrol per lamb daily significantly increased total gain.

The implantation of either diethylstilbestrol or estradiol plus progesterone, or the oral administration of 4 mg. dienestrol daily produced a marked increase in feed efficiency ($P<0.01$) and the feeding of 2 mg. of dienestrol daily improved feed efficiency ($P<0.05$) although it did not significantly improve gain. The combination of 2 mg. of dienestrol and 25 mg. of methyl testosterone orally also improved feed efficiency ($P<0.01$).

Mammary stimulation was produced by all treatments in which which diethylstilbestrol, dienestrol, or estradiol were included.

Total slaughter loss—the difference between liveweight at Purdue and cold carcass weight—was greatest in the lambs which received, (1) the estradiol-progesterone pellets, (2) 4 mg. of dienestrol per day orally ($P<0.01$), and (3) the implantation of 12 mg. diethylstilbestrol, ($P<0.05$).

The subcutaneous implantation of diethylstilbestrol, or estradiol plus progesterone, or the oral administration of dienestrol alone or in combination with methyl testosterone did not produce carcasses of as high a grade as the control lambs.

The estrogenic activity of the meat of estrogen-treated lambs was not significantly different from the controls, with the exception of a slight increase in estrogenic activity of a lamb implanted with estradiol plus progesterone. The oral administration of dienestrol at levels of 2 or 4 mg. per lamb daily, alone or in combination with methyl testosterone, produced a highly significant increase in the estrogenic activity of the liver.

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FACTORS AFFECTING SEDIMENTATION AND MOTILITY OF RAM SPERMATOZOA [1]

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THE sedimentation of sperm stored under various environmental conditions has received little study. Work has been reported by Kampschmidt et al. (1951) and Szumowski (1948). However, these studies were made, in most cases, at storage temperatures in which the motility of the spermatozoa was greatly suppressed and the concentrations of spermatozoa were not standardized.

The purpose of these studies was to develop a simple technique for measuring the sedimentation of sperm and to investigate factors affecting sedimentation and its relation to motility in ram spermatozoa.

Materials and Methods

The concentration of ram spermatozoa within certain limits was found to be directly proportional to the optical density of diluted semen samples as determined with the electrophotometer. Thus, the amount of change in optical density for a given period of time would likewise be directly proportional to the number of sperm cells sedimenting out of the portion of the column through which the light is transmitted. Therefore determination of the change in optical density per unit time constitutes a simple, rapid method for estimating the sedimentation rate of spermatozoa.

The AC model Fisher electrophotometer with a green filter having a wave length of approximately 525 millimicrons was used to measure optical density in this study. Any variation in the color of the seminal plasma or the amount of contaminants present in the semen, which introduce error into the electrophotometric determinations of sperm concentration will introduce error into these results. However, no marked differences were observed in these regards.

Preliminary studies showed that both the interval between collection and dilution and the temperature during that interval have an important effect on subsequent sperm motility (estimated percentage of sperm

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showing forward motion). Temperatures of 21° and 5° C. (each attained by slow initial cooling) and intervals of 1, 2, 3 and 4 hours were studied. The 21° C. temperature and 4-hour interval gave the best motility in each instance. This latter observation on the longer interval being advantageous is in agreement with the results of a study made by Madsen, 1953. Also, the rate of change in optical density during sedimentation appeared to be increased as predilution interval and temperature were varied so as to increase motility.

Experiment I

Experiment I (table 1) was designed to test the effect of different temperatures during the test on sedimentation rate and to describe the sedimentation curve during a 5-hour interval.

Semen samples were collected from each of two rams on two different days. Dilution was begun approximately 2 hours and 15 minutes from the time of collection. Two replicate dilutions were made for each ram on each day by diluting the semen with a 0.1 molar sodium citrate solution to an optical density of 70. Thus, there was a total of eight replicate dilutions in all. Three electrophotometer tubes (A, B and C) were filled to a standard depth from each of the eight replicate dilutions, making a total of 24 tubes.

Three test tube racks were provided. One was kept at air temperature, which ranged from 25° to 29.2° C., while the other two racks were kept in water baths. One of these baths was maintained at 34° C. and the other was maintained at 37° C.

At about 50 minutes from the beginning of dilution one tube from each replicate dilution after being gently agitated was placed in each rack under the three different temperatures; i.e., the eight A tubes were put in the test tube rack kept at 25° to 29.2° C.; the eight B tubes were put in the test tube rack kept at 34° C.; the eight C tubes were put in the test tube rack kept at 37° C. At two and again at three, four and five hours from the beginning of sedimentation the electrophotometer tubes, representing the various temperatures and dilutions, were carefully taken from the water baths (those not in water were dipped in water), the excess moisture was wiped from them and the optical density readings were obtained. As soon as the readings were obtained the tubes were carefully returned to their respective temperature treatments until the final reading was obtained.

The rate of change in optical density per hour was calculated by dividing the difference between a particular optical density reading

TABLE 1. THE EFFECTS OF TEMPERATURE ON RATE OF CHANGE IN OPTICAL DENSITY[a] OF DILUTED RAM SEMEN SAMPLES DURING SEDIMENTATION

			Temperatures											
			25–29° C. (A tubes)				34° C. (B tubes)				37° C. (C tubes)			
			Sedimentation time (Hr.)				Sedimentation time (Hr.)				Sedimentation time (Hr.)			
Ram	Day	Replicate dilutions	2	3	4	5	2	3	4	5	2	3	4	5
24	I	1	10.9	10.5	1.5	−0.5	7.4	3.7	2.1	0.7	6.2	3.1	0.0	1.6
		2	8.8	16.1	1.0	0.0	10.2	−0.7	2.7	0.6	6.2	0.8	1.6	0.7
	II	1	10.6	1.3	0.0	1.9	6.1	1.7	0.8	1.6	4.9	0.8	0.8	0.8
		2	10.9	0.0	0.7	0.7	6.5	1.7	0.8	0.8	6.1	0.8	0.9	0.0
30	I	1	12.2	4.7	1.0	0.0	9.6	−0.7	0.7	1.4	7.0	−0.8	0.8	1.5
		2	15.6	1.0	−0.5	0.5	9.9	0.0	0.0	0.2	6.5	−0.5	1.4	0.8
	II	1	9.2	2.1	0.0	0.7	6.1	0.0	0.0	0.8	4.0	1.8	0.0	0.8
		2	8.9	3.4	1.3	1.2	7.0	0.8	0.0	1.5	4.4	1.7	0.8	0.0
Average			10.9	4.9	0.6	0.6	7.8	0.8	0.9	1.0	5.7	1.0	0.8	0.8

[a] Change in optical density per hour.

for a tube and the preceding optical density reading for that tube, by the number of hours which had elapsed between the two readings.

Experiment II

Experiment II (table 3) was designed to test for differences in sedimentation rate between rams. Also, previous experiments had suggested an association between rate of change in optical density and motility and an attempt was made to measure this association when

TABLE 2. ANALYSIS OF VARIANCE FOR THE EFFECT OF TEMPERATURE DURING SEDIMENTATION ON CHANGE IN OPTICAL DENSITY PER HOUR IN A FIVE-HOUR SEDIMENTATION TEST

Source of variation	Classification of Main variable	D.F.	Mean square	Error Term No.	F
Within rams and days		92			
1. Temperature (Te)	Fixed	8	11.94	6	34.1**
2. Time (Ti)	Fixed	12	82.39	5	147.1**
3. Replicate (Rep.)	Random	4	.40		
4. Te×Ti		24	9.80	7	4.9**
5. Ti×Rep		12	.56		
6. Te×Rep		8	.35		
7. Ti×Rep×Te		24	1.99		

** $P<0.01$.

motility variations were produced by differences in post dilution interval both before and during sedimentation.

Semen collections were obtained from three rams (group I) on the first day and from the same three rams again on the second day. More collections were obtained from another five rams (group II) on the third day and from the same five rams again on the fourth day. Thus, 16 separate semen samples were obtained, two from each of eight rams. At three hours from the time of collection in each instance two replicate dilutions were made from each sample. The samples were diluted to an optical density of 70 with a 0.1 molar sodium citrate solution as in the previous experiment.

Four electrophotometer tubes (A, B, C and D) were filled to a standard depth from one of the two replicate dilutions and two tubes (B and D) from the other replicate dilution with each group, day and ram. The optical density was again checked.

At 30 minutes from the beginning of dilution the A and B tubes were gently inverted several times and placed in a perpendicular posi-

TABLE 3. THE EFFECTS OF TIME FROM DILUTION TO THE START OF SEDIMENTATION ON SEDIMENTATION RATE[a] AND MOTILITY IN RAM SPERMATOZOA DILUTED IN A SODIUM CITRATE SOLUTION

			Time from dilution to sedimentation									
			30 Minutes					60 Minutes				
			A tubes Motility[c] after the following No. Hr. of sedimentation				B tubes Rate of change in Opt. D.[a]	C tubes Motility[c] after the following No. Hr. of sedimentation				D tubes Rate of change in Opt. D.[a]
Group	Day	Ram	0	1	2	3		0	1	2	3	
I	1	29	55[d]	40	17.5	5	8.43[e]	50[d]	25	5	.5	7.62
		30	60	55	35.0	7.5	10.76	55	45	20	5	10.83
		1	45	30	7.5	1	11.52	40	20	4	.5	10.07
	2	29	60	40	30	7.5	11.02	55	20	15	4	9.02
		30	65	50	35	20	13.00	55	35	25	7.5	11.93
		1	60	45	30	7.5	10.68[b]	55	40	15	4	8.63[b]
II	1	12	37.5	22.5	5	.5	5.78	32.5	17.5	1	.5	4.62
		8	55	55	27.5	7.5	10.60	55	40	22.5	5	9.48
		32	2	5	.5	0	3.10	20	5	1	.5	2.63
		17	65	55	25	5	10.68	57.5	35	30	10	9.97
		6	60	40	40	30	13.22	55	40	40	20	13.52
	2	12	32.5	17.5	5	1	3.75	....	....	....	....	3.56
		8	60	40	30	10	8.73	55	40	22.5	5	9.48
		32	10	5	5	5	2.67	5	5	5	.5	2.43
		17	65	40	30	10	10.17	57.5	35	30	10	9.94
		6	60	30	20	7.5	8.42	55	25	15	5	8.02

[a] Change in optical density per hour in a three-hour sedimentation test.
[b] Some agglutination of cells occurred in these preparations.
[c] Estimated percentage of sperm cells showing forward motion.
[d] Estimates of motility made on A and C tubes from 1 replicate only.
[e] Average of determinations made on B tubes from both replicates or on C tubes from both replicates.

tion in a water bath kept at approximately 29° C. At 60 minutes from the time that each sample was diluted the C and D tubes were similarly agitated and placed in the water bath.

An estimate of motility (percentage of cells showing forward motion) was obtained from the A and C tubes just prior to the time that they were placed in the water bath and again at one, two and three hours of sedimentation. As in experiment I, the sperm cells were redispersed at each of these periods prior to the obtaining of a few drops for microscopic examination.

At three hours from the beginning of sedimentation the optical density reading was obtained from the B and D tubes for each replicate (the values for rate of change in optical density obtained from the B and D tubes have been averaged for simplicity in table 3).

Experiment III

Experiment III was done to investigate the reason for the close association between rate of change in optical density and motility. Semen samples from eight rams were diluted to a standard optical density of 70 and sedimented in a water bath kept at approximately 29° C. At between 2 and 2.5 hours from the beginning of sedimentation an estimate of motility was obtained from sperm cells still suspended in the upper half (floating fraction) of the tubes, and another estimate of sperm motility was obtained from the thick, sedimented layer (sedimented fraction) in the bottom of these tubes (table 6). The latter sperm were obtained by means of a pipet and diluted while on the microscope slide to roughly the same concentration as the cells obtained from the upper fraction.

Statistical Methods

The assumptions made as to the classification of the variables (random, or fixed) in the various analysis of variance tests and the error terms used are shown in the analysis of variance tables.

The error terms have been selected by determining the variance components for each mean square according to a "rule of thumb" method devised by Henderson (1954).

Results and Discussion

Experiment I

Both temperature and the time interval had highly significant effects on the rate of change in optical density (table 2). There was also a

highly significant interaction of temperature × time. These effects may be seen readily by inspection of the data (table 1). The temperatures above room temperature (25° to 29° C.) depressed the sedimentation rate (rate of change in optical density) but it may be seen also that it was only during the first two hours of sedimentation that temperature had a noticeable effect on the sedimentation rate.

The sedimentation rate was the most rapid during the first two hours of sedimentation, was somewhat slower during the third hour, and had leveled off at a rather slow rate during the fourth and fifth hours. Earlier observations in a preliminary experiment (table not shown) indicated that sedimentation continues to occur at a slow rate for many

TABLE 4. ANALYSIS OF VARIANCE FOR THE EFFECTS OF RAM AND TIME ON THE CHANGE IN OPTICAL DENSITY PER HOUR DURING A THREE-HOUR SEDIMENTATION TEST

Source of variation	Classification of Main variable	D.F.	Mean square	Error Term No.	F
Within group		62–6=56			
1. Time (Ti)	Fixed	2	5.07	5	14.1**
2. Rams (R)	Random	6	77.60	3	8.2**
3. Days (D)/R	Random	8	9.51	4	59.4**
4. Repl/D/R		16	.16	7	1.6
5. R×Ti		6	.36	6	.6
6. D/R×Ti		8–1=7	.59	7	5.9**
7. Repl/D/R×Ti		16–5=11	.10		

** $P<0.01$.

hours. The average rate of change in optical density during the first three hours in those earlier observations was approximately 11 per hour but was slightly over 1 for 19 additional hours.

Experiment II

There was a highly significant difference ($P<.01$, table 4) in the rate of change in optical density between rams within ram groups (table 3). The change in optical density within groups of rams was more rapid when sedimentation was begun at 30 minutes (time) after dilution than when it was begun at 60 minutes after dilution ($P<.01$).

A highly significant difference was found between days within rams. It seems reasonable to assume that the day-to-day variations in temperature, though not nearly so extreme as those studied in the preliminary studies, might have contributed an important part to this between-day variation.

The correlations of motility, at the start of sedimentation and after one, two and three hours of sedimentation, with rate of change in optical density through a three-hour interval (when sedimentation was begun 30 minutes after dilution) were $r=0.890$, $r=0.868$, $r=0.847$, and $r=0.620$, respectively. The correlations of motility, at the start of sedimentation and after one, two and three hours of sedimentation, with the rate of change in optical density through a three-hour interval (when sedimentation was begun 60 minutes after dilution) were $r=0.855$, $r=0.833$, $r=0.782$, and $r=0.702$, respectively. All of the above correlations were highly significant ($P<.01$). These two sets of correlations were tested for heterogeneity and were not found to be heterogeneous. The pooled correlations for each set of four correlations were compared and were found not to differ significantly.

The between-ram correlations between motility at the start of sedimentation and rate of change in optical density through a three-hour interval, and between motility at one hour of sedimentation and the rate of change in optical density through a three-hour interval (when sedimentation was begun 30 minutes after dilution) were $r=0.949$ ($P<.01$) and $r=0.923$ ($P<.01$) respectively; i.e., the rams with higher percentages of motility tended to have higher sedimentation rates, as reflected in the rate of change in optical density. The within-ram correlations ($r=0.280$ and $r=0.381$) were not significant. However, there were only two observations per ram and little variation within each ram. Also, much of this within-ram variation was probably due to technical error with some additional variation due to differences between the two consecutive days on which the tests were made.

Experiment III

The average motility of sperm from eight rams obtained from the upper part of the tubes (table 5) after 2 to 2.5 hours of sedimentation was 6.6% as compared with 52.6% in the thick, sedimented layer. From an inspection of the data it is obvious that this difference is real. This is not in agreement with Kampschmidt *et al.* (1951) who found very little difference in motility and viability between the sedimented and floating fraction. However, it should be pointed out that sedimentation occurred at 4° to 7° C. in their study and not at room temperature. They suggested that sperm behave as inert particles at this temperature.

At least two postulates for this difference in motility between the two different fractions might be suggested:

1. The motility might be more rapid in the sedimented layer because the motile sperm had the ability to get to the bottom faster on the average than the immotile sperm.

2. Motility might have been better preserved in the sedimented layer because of the concentration; whereas those which by chance did not

TABLE 5. DIFFERENCES IN MOTILITY BETWEEN THE FLOATING AND SEDIMENTED FRACTIONS OF SEMEN SAMPLES AFTER 2 TO 2.5 HOURS OF SEDIMENTATION

Ram	Percent motility	
	Floating fraction	Sedimented fraction
29	12.5	65
30	10	65
1	7.5	70
12	4	45
8	5	55
32	1	1
17	5	60
6	7.5	60
Average	6.6	52.6

get to the bottom lost most of their motility because of a deleterious effect of the dilution.

Postulate 1 seems to be consistent with other observations given above. If it is true that motile sperm cells do descend through the medium more rapidly than immotile sperm cells, what is the explanation? A study made by Barer *et al.* (1953) indicates that the various parts of human sperm cells might vary in density; the heads having a much greater specific density than a cytoplasmic collar just posterior to the head. This suggests that a difference in the rate of sedimentation between the two types of cells might be due to a physical orientation of the sperm cells in the less dense medium with the head lower than the tail due to a difference in relative density of the head as compared with the density of all structures posterior to the head. If a sperm cell, oriented in such a way, were propelled, it would certainly reach the bottom much sooner than a non-motile sperm cell. However, this phenomenon might just as easily be explained by postulating a positive geotropism, a chemotaxis, or some factor which causes sperm to "flock together."

Further evidence indicates that there may also be some advantage in the maintenance of motility for those sperm cells in the closely packed

layer (postulate 2). This comes from the observation that the motility of cells obtained from the sedimented layer at 2 and 2.5 hours after the start of sedimentation was as high or higher than the motility observed at the start of sedimentation; whereas motility was somewhat lower at two hours from the beginning of sedimentation in those samples in which sperm cells were redispersed at each hour. The validity of this assumption is somewhat dependent upon the relative proportions of the total number of sperm cells which might be found in the two fractions at the time of observation. This is in general agreement with observations made by Cheng *et al.* (1949) who reported a progressive decrease in the percentage of motile sperm from low to high dilutions.

The sperm from one semen sample showed considerable agglutination which appeared to increase markedly the rate of change in optimal density. Szumowski (1948) also observed that the clumping together (agglutination) of sperm cells greatly increases the rate of sedimentation.

Discussion

Observations made in this series of experiments suggests the hypothesis that the sedimentation rate of ram spermatozoa is a reflection of the percentage of sperm cells that is motile and the degree of endurance of that motility during this period, plus a passive type of sedimentation which is entirely independent of motility and occurs throughout this period and continues to occur at a slow rate even after motility ceases.

A summary of the evidence for this hypothesis is as follows:

1. The sedimentation rate of sperm cells has been modified by exposing the cells to different predilution temperatures, by aging the sperm before dilution and by varying the time from dilution to sedimentation. Ram differences have also been a modifying factor. Sedimentation rate (irrespective of treatment) was always found to be correlated with motility.

2. Motility at zero, one, two and three hours of sedimentation and rate of change in optical density through a three-hour interval (sedimentation rate) are highly correlated. Further, the between-ram correlations between motility at the start of sedimentation and the rate of change in optical density through a three-hour interval, and between motility at one hour of sedimentation and the rate of change in optical density (when sedimentation was begun 30 minutes after dilution) were $r=0.949$ ($P<.01$) and $r=0.923$ ($P<.01$) respectively.

3. The percentage of motile sperm taken from the sedimented layer is much higher than that from the supernatant fraction.

4. Evidence for a passive type of sedimentation which accompanies that associated with motility may be inferred from the fact that though sedimentation is rapid when motility is high, sedimentation continues at a slow rate even after motility is completely lost in the suspended cells. Further, Kampschmidt *et al.* (1951) found that only about 58% of spermatozoa, which had been killed by temperature shock, remained in suspension after 24 hours of storage at 4° to 7° C. in an egg yolk citrate diluent.

The standardizing dilution rate was about 50 to 1 in an average sample. Inasmuch as sedimentation occurred normally at these high dilution rates in well buffered diluents (Krebs-Ringer and sodium citrate) it seems unlikely that a change in pH during sedimentation had any important effect on sedimentation rate.

It is also doubtful that variations in viscosity of the seminal plasma would have an important effect at these high dilution rates.

Lindahl *et al.* (1952) observed an increase in the specific density of bull spermatozoa with ripening. Inasmuch as only first and second ejaculates were used in these experiments and since the rate of sedimentation was too rapid to be accounted for (to any great extent) by passive sedimentation it appears unlikely that variations in specific density would have any important influence on these results.

If the proposed hypothesis is true, then optical density obtained in a standard way might be a method of obtaining objective measures of sperm motility.

Summary

The effects of predilution temperature, age of sperm at dilution, length of time from dilution to sedimentation, temperature during sedimentation, and individuality of ram on rate of change in optical density (sedimentation rate) of spermatozoa, and the correlation of sedimentation rate and motility have been studied.

The semen was diluted to a standard sperm concentration (optical density of 70) in a 0.1 molar sodium citrate solution by use of an electrophotometer using a green filter with a wave length of approximately 525 millimicrons. Following a given period of sedimentation another optical density reading was obtained, and the rate of change in optical density per hour was then calculated.

Semen kept at 21° C. prior to dilution and sedimentation had a significantly more rapid sedimentation rate than when kept at approximately 5° C. ($P<.01$).

Also, with increase in sperm age at dilution up to four hours, there was a significant increase in sedimentation rate ($P<.01$).

With increase in time from dilution to the start of sedimentation there was a significant decrease in sedimentation rate ($P<.01$).

Temperatures above room temperature during sedimentation depressed sedimentation rate ($P<.01$).

Sedimentation rate was most rapid during the first two hours of sedimentation, then dropped off sharply but continued at a slow rate for many hours.

The differences in sedimentation rate between rams was significant ($P<.01$).

The sedimented semen fraction had a greater percentage of motile sperm than did the floating fraction.

The total and between-ram correlations between rate of change in optical density and motility were high ($r=0.923$ and 0.949) and significant ($P<.01$). Sedimentation rate appeared to be positively associated with motility, irrespective of treatment.

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NEWS AND NOTES

Nominations for the Morrison Award. All nominations in writing are due in the Secretary's office by *June 15*. The rules governing this award are stated in the JOURNAL OF ANIMAL SCIENCE, Vol. 11, pp. 807–809. All nominations must be accompanied by a brief biography of the nominee, a list and reprints of publications concerning the research, and a statement of the importance of this research to the livestock industry and animal science. The award for 1956 will be $1,250 and a gold watch.

Nominations for the American Feed Manufacturer's Award. The rules governing this award are as stated in the JOURNAL OF ANIMAL SCIENCE, Vol. 7, pp. 257–258. No formal nominations for the membership are required, although the Award Committee would appreciate receiving nominations. These should also be sent by *June 15* to the Secretary's office. Research published in 1954 and 1955 will be considered for the 1956 award.

1956 Annual Meeting. The annual meeting will be held at Hotel Sherman, Chicago, on November 23 and 24. You will be sent a questionnaire in early June regarding papers and titles which are to be submitted to the Secretary's office by July 20. Abstracts of papers are to be submitted by August 15. Only members of the Society can submit papers, but associate authors can be non-members.

The joint annual meeting of the Western Section, American Society of Animal Production and the Western Division, American Dairy Science Association, will be held at the University of Nevada, Reno, July 16 to 18, 1956.

K. L. Durrance, a University of Florida graduate and formerly County Agent was appointed Swine Specialist at the University of Florida on December 1, 1955.

L. N. Hazel, Professor of Animal Husbandry, Iowa State College, was on leave from about March 31 to May 1 to assist in planning research programs at three new animal science centers in Colombia, South America. This work is supported by a grant from the Rockefeller Foundation.

Glenn H. Beck, former Head of the Dairy Husbandry Department, University of Maryland, has been appointed director of Kansas Agricultural Experiment Station.

R. T. Clark, regional coordinator of the Western Regional Beef Cattle Breeding Project, has been granted a Fulbright Award for post-graduate studies in Australia where he will work with representatives of the Commonwealth Scientific and Industrial Research Organization on performance testing of beef cattle. Dr. Clark plans to leave for Australia about March 15, and will be gone for a period of 6 or 7 months.

Carl F. Sierk resigned as Head of the Animal Husbandry Department, University of Idaho, on February 1 to become Assistant Chief of the Animal Husbandry and Poultry Branch, International Cooperation Administration, Washington, D. C.

R. H. Lush, Professor of Dairy Husbandry, Tennessee Agricultural Experiment Station, is on a 2-year leave of absence to serve as pasture, forage, and livestock adviser in Brazil under an International Cooperation Administration assignment.

J. A. Bennett, Head of the Animal Husbandry Department at Utah State Agricultural College, is on a 6-months leave for graduate work in animal breeding at the University of Minnesota.

Howard W. Johnson was appointed Chief of the Animal Disease and Parasite Research Branch of the U. S. Department of Agriculture's Agricultural Research Service, effective January 30. Dr. Johnson had been serving as acting Chief of the Branch since September 1 when Dr. B. T. Simms, former Chief, was appointed Director of Livestock Research for the Agricultural Research Service.

George R. Johnson is now Associate Professor in the Animal Science Department at Ohio State University where he will engage in teaching and research on types of sheep and systems of management in producing market lambs. Dr. Johnson is a Cornell graduate and obtained his doctorate at Michigan State University.

O. F. Pannish, Assistant Professor of Animal Husbandry, University of Arizona, is on a year's leave of absence for graduate study in animal breeding at Oregon State College.

O. Glenn Hall, Associate Animal Husbandman, and Ira L. Dyer, Assistant Animal Husbandman, are recent additions to the Animal Husbandry staff at the University of Tennessee.

Robert P. Moorman, Associate Animal Husbandman, University of Tennessee, is now in charge of the livestock program at the Ames Plantation, Grand Junction, Tenn.

L. E. Harris, Professor of Animal Husbandry and Chairman of the Institute of Nutrition, Utah State Agricultural College, Logan, is on a year's sabbatical leave to undertake research on sheep nutrition in Australia under a Fulbright scholarship. He and his family expect to return to Logan about February 15, 1957.

The following additions have been made to the staff of the Animal and Poultry Husbandry Research Branch, Agricultural Research Service, at Beltsville, Md.:

Elbert F. Johnston joined the staff of the Swine Research Section as a geneticist in August 1955, after completion of his Ph.D. work at Oregon State College. He is devoting a major portion of his efforts to gene-environment interaction studies with swine.

James Bond joined the Cattle Research Section as an Animal Husbandman in August 1955, following completion of his work for a Ph.D. at the University of Illinois. A major portion of his present work is being devoted to a study of the effect of jet aircraft sound on production and reproduction of livestock. The project is being conducted in cooperation with the U. S. Air Force. A native of Colorado, he took his under-graduate work at the University of Illinois and his M.S. in animal production at the University of Wyoming.

James N. Wiltbank joined the staff of the Cattle Research Section as a geneticist in September 1955, following the completion of his work for a Ph.D. at the University of Wisconsin. He is a native of Arizona and a graduate of Brigham Young University. He is studying the physiology of reproduction of sheep and dual-purpose cattle.

J. Lane Fletcher joined the staff of the Meat Production and Quality Research Section as a geneticist on February 1, 1955, after completion of his Ph.D. work in genetics at Texas A. & M. College. A native of Louisiana, he took his undergraduate work at Southwestern Louisiana Institute, Lafayette, and his M.S. in Dairy production at Texas A. & M. College. He is devoting major efforts to the study of immediate pre-slaughter treatments on carcass composition and quality.

C. D. Lowe, 73, passed away Friday, December 23, 1955, following a month's illness after a cerebral hemorrhage. He had served 26 years as an Extension Animal Husbandman in the Federal Extension Service and the Bureau of Animal Industry, retiring from active service June 30, 1951. Dr. Lowe was the recipient in 1951 of the Superior Service Award of the U. S. Department of Agriculture for his exceptional understanding, ability, and leadership in fostering and developing a cordial, effective relationship between the veterinary profession and the various educational institutions; for blending that cooperative attitude with research results as he made them available to breeders, livestock producers, marketing agencies and meat packers; and for pioneering practical, healthy, effective production as the goal of all members of the livestock industry.

A cooperative graduate summer session in statistics at North Carolina State College on June 11–July 20, 1956 will be held jointly with the Institute in Quantitative Research Methods in Agricultural Economics, sponsored by the Social Science Research Council. Several statistics courses will be oriented towards economic applications. Inquiries should be addressed to: Professor J. A. Rigney, Department of Experimental Statistics, North Carolina State College, Raleigh, North Carolina.

The 1956 summer school of the Department of Statistics at Iowa State College will include six courses at the graduate minor level for students majoring in other subject-matter fields; these courses are also designed to satisfy background requirements for graduate major work in statistics. The offerings will be given in two sessions: June 11–July 18 and July 18–August 24. Students may register for either six-week session or for the full 12-week summer quarter. Additional information may be obtained from T. A. Bancroft, Director, Statistical Laboratory, Iowa State College.

INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, doubled-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should consist only of a paragraph indicating very briefly the nature of the question asked and the reason for asking it. Reference to relevant literature should be made later in the discussion of the results. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results or their relation to some problem of the livestock industry. Each paper should end with a brief summary in the form of an abstract containing the most pertinent data in the briefest possible form.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. There will be a charge for making engravings in excess of one page per paper and of printing tables in excess of two pages per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual that will assume responsibility for the charges and for the reprints ordered at that time.

6. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts." *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest to members. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. J. K. Loosli, Department of Animal Husbandry, Cornell University, Ithaca, New York, or to the publishers: Boyd Printing Company, 372–374 Broadway, Albany 7, New York.

12. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, Dr. W. M. Beeson, Department of Animal Husbandry, Purdue University, Lafayette, Ind., or to the publishers, Boyd Printing Company, 372–374 Broadway, Albany 7, New York.